Infection, immunity and island-life

Tieleman, Bernadine; Matson, Kevin; Vergouwen, S.; Horrocks, Nicholas

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Centre for Ecological and Evolutionary Studies (CEES)

Address: Centre for Life Sciences
PO Box 11103
9700 CC Groningen
The Netherlands
tel. +31 (0)50 363 8357
Fax +31 (0)50 363 2295
Email: CEES-office@rug.nl
http://www.rug.nl/cees

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Highlights

Out of the old and into the new

In December 2010 CEES researchers said farewell to the Biological Centre in Haren. After some 40 years the old building was outdated and a renovation was not considered feasible due to asbestos and a 1960s building design that was definitely not eco-friendly. We have fond memories of the lovely wooded location next to the *Hortus* but times have changed. Our new quarters in the hypermodern Centre for Life Sciences is something to behold. Located in the Zernike complex on the north side of the city, this signature building combines state-of-the-art laboratory facilities and an eco-friendly power plant with a light and inviting ambience.

Irene Tieleman wins VIDI grant

Animal Ecology Group member Irene Tieleman won a VIDI grant on 'The impact of environment on avian immunity: ecology and evolution of the match between microbial communities and antimicrobial defenses' The central question addressed in the proposed new work is: 'How do animals adjust their immune system - central to protection against pathogens and diseases – to environmental change?' It is widely assumed that animals should match their immune system to the threats they face, because immune function is costly. But, little is known about these threats. How do pathogen diversity and abundance differ among environments and seasons? Which pathogens are most important? This project aims to address these questions, *crucial* to place immune defenses in an ecological and evolutionary context. We will exploit an elegantly simple and widespread avian model, the 'egg-nest' dyad, to study the association between antimicrobial defenses of eggs and microbial communities in their environment. This first attempt to map microbial and pathogen landscapes and concurrent avian responses, opens up important new avenues to understand selection pressures on vertebrate immune function, individual traits related to disease resistance, and their relationships with distributions and numbers of animals in a changing world.

A boost for the marine biology cluster

Prof. Dr Per Palsbøll formally joined the CEES institute in the Spring of 2010. He will lead the new *basiseenheid* Marine Evolution and Conservation (MarECon). Per's research is focused on long and short-term processes that structure marine mammal populations, such as environmental and geological changes, migratory behavior and community effects. His research also aims to develop novel analytical approaches to infer migration rates and abundance of whales from genetic assessment of extended-kinship. His group is also involved in several genome sequencing projects of selected marine mammal species. Important fields of application of this work are evolutionary ecology and the conservation and management of marine mammal populations.

Per earned his PhD in evolutionary genetics from the University of Copenhagen (1993). Following post-docs in the US and UK, he was hired as an Assistant Professor at the Department of Environmental Science, Policy and Management at the University of California-Berkeley in 2001 and tenured in 2006. Wishing to return to Europe, he was hired as Full Professor in the Department of Genetics, Stockholm University in 2007 before accepting the position here in Groningen. At present he is on a year-long sabbatical at Harvard and will start in September 2011.

A top year for Evolutionary Genetics

2010 was an absolute top year for Evolutionary Genetics. It all started in January with seven publications within one month. One of these was the official publication of the Nasonia Genome Project in *Science* (Werren *et al.* 2010). During the international *Drosophila* conference in Washington in 2004 the idea was raised to sequence the complete genome of our model organism, the parasitic wasp *Nasonia*. As a follow up we wrote the required White Paper (official proposal) with American colleagues at a meeting in Groningen. After approval by the National Institute of Health (NIH), the Baylor College of Medicine in Houston performed the actual sequencing work. An international consortium of scientists subsequently annotated the genome which resulted in the publication in Science in January 2010. Simultaneously with this publication two special editions appeared on *Nasonia* research, one in *Heredity* and one in *Insect Molecular Biology*. Our group participated in four publications in these special issues.

Sex determination in Science and success with a TOP-subsidy

In a publication in *Science* and based on her PhD work under supervision of Louis van de Zande, Eveline Verhulst describes the sex determination mechanism of *Nasonia*. This entailed the elucidation of a completely new mechanism of sex determination and meant the solution of a long-standing open question since the middle of the last century. As a follow-up we were invited to write a review on insect sex determination in Current Opinion in Genetics & Development. Later in the year Leo Beukeboom received a TOP grant from the Netherlands Organisation for Scientific Research (NWO) to continue this research. Group members contributed to a popular Cahier Biowetenschappen and Maatschappij .

Control of plant mineral nutrition

A FP7 Marie Curie Training Network grant "*Biochemical and Genetic Dissection of Control of Plant Mineral Nutrition* (BIONUT-ITN)" was awarded to an international collaboration initiated by Luit de Kok (Ecophysiology of Plants). The grant is aimed at providing state-of-the-art training for young researchers in the highly strategic field of plant nutrition through an international program of research, training and transfer of knowledge. The overall goal of the network is increasing the understanding of genetic control of nutrient use efficiency of plants and thus addressing global food security. The grant, with an EU contribution of 2.42 million euros, covers the appointment of 8 PhD students and 2 post-docs. The BIONUT network includes research groups in Poland, France, Italy, the UK, Germany, Belgium and The Netherlands.

Chris Smit is the new tenure-tracker at COCON

Dr. Christian Smit joined the CEES institute on 1 october 2010 for a tenure-track position with the Community and Conservation Ecology group. His research focuses on positive species interactions and consequences for the dynamics and spatial organization of plant communities in human dominated and natural environments. This typically involves large-scale field experiments in herbivore driven ecosystems to study the impact of facilitation by the so called 'nurse plants' on protégé species. Study sites are situated in the Jura Mountains

of Switzerland, in Mediterranean oak savannas (dehesas) in Spain and in grazed nature reserves in The Netherlands. With this research he hopes to contribute to the advancement of fundamental ecological concepts. One of these concepts is the 'stress-gradient-hypothesis'which predicts that the nature of species interactions switches from competitive to facilitative with increasing environmental stress. Of special interest in his work is now the question how plant-plant interactions change along stress gradients consisting of both abiotic stress and biotic stress (herbivory), focussing on salt marshes and coastal dunes at the Wadden Sea area and (semi)arid steppes in Southern Spain.

Zuidelijke reuzenstormvogel. Foto Jeroen Creuwels

Sneeuwval nekt stormvogel

Toegenomen sneeuwval in het Zuidpoolgebied bedreigt indirect broedende Arctische stormvogels. De reuzenstormvogel kan door de sneeuw onverhoeds toeslaan vanaf de grond.

Door onze redacteur
SANDER VOORMOLEN

ROTTERDAM, 7 DEC. Sneeuw is de aartsvijand van de Antarctische stormvogel. Maar het is niet de hou die deze geharde zuidpoolvogel parten speelt, ontdekte biologe Jeroen Creuwels. Nee, de oorzaak blijkt de Zuidelijke reuzenstormvogel die jacht maakt op de broedende stormvogels. Alleen als er sneeuw ligt, kan deze uit de kluiten gewassen vogel landen in de broedkolonie op de met rotsblokken bezaaide kliffhellingen en zijn slag slaan.

De gevolgen voor de Antarctische stormvogel zijn desastreus, ontdekte Creuwels, die vrijdag in Groningen op zijn onderzoek hoopt te promoveren. Van de 29 eieren die Creuwels telde in oktober, aan het begin van het broedseizoen, vloog slechts één kuiken uit. De Zuidelijke reuzenstormvogel was grotendeels verantwoordelijk voor die dramatisch slechte broedsucces. Deze grote vogels, die wel vijf kilo wegen en grote stijve vleugels hebben, kunnen normaal niet landen op stelle rotshellingen.

Maar als er voldoende sneeuw ligt, kunnen ze een zachte landing maken en zich gewoon laten vallen. Vervolgens lopen ze gewoon de kolonie in.

Creuwels zag dat de Antarctische stormvogels aanvankelijk wel opvlogen met zo'n jager in hun midden, maar dat zij na een tijdje leken te zijn vergeten dat die er was. „De reuzenstormvogel liep de kolonie in, en hield zich dan een kwartier, soms wel uren, gedeisd. Met zijn kop in zijn veren leek hij wel een steen. Maar dan sprong hij plotseling op en greep een nietvermoedende stormvogel."

In de paniek raakt de Antarctische stormvogel ook nog bijna al zijn eieren kwijt, omdat nog andere vogels, Zuidpooljagers, onmiddellijk toeslaan. De kleine vogels roven onmiddellijk de onbewaakte eieren weg zodra de ouders opvliegen. De reuzenstormvogel zelf laat de eieren links liggen.

Creuwels deed zijn ontdekking op Ardery Island, een klein rotsachtig eilandje dat op enkele kilometers afstand ligt van het vasteland van Antarctica, aan de kant van Australië. Sneeuwbanken hoeven er niet eens te ontstaan door sneeuwval op het eiland zelf, hardere winden blazen de sneeuw ook van het continent het eiland op.

Het jaggerdrag van de reuzenstormvogel was nog niet eerder waargenomen. De vogel stond bekend als aaseter, de aasgier van de Zuidpool, die zich voornamelijk voedde met dode jongen van de Weddellzeehond en dode pinguïns. Creuwels: „We wisten dat hij ook wel eens stormvogelkuikens rooft, maar dat hij ook volwassen vogels te grazen neemt was niet eerder gezien."

De effecten op de populatie Antarctische stormvogels zijn groot. In het seizoen 1996-'97, toen er veel sneeuw lag, viel 15 procent van de geringde volwassen vogels ten prooi aan de reuzenstormvogel. De normale sterfte bedraagt slechts 4 procent per jaar.

Hoe het nu gaat met stormvogels op Ardery weet Creuwels niet. Het onderzoek is gestopt nadat hij er vertrok. Creuwels heeft er vervolgens tien jaar over gedaan om zijn proefschrift af te ronden. Omdat zijn beurs was opgehouden, moest hij er in zijn vrije tijd aan werken terwijl hij daarnaast werkte om in zijn levensonderhoud te voorzien. De bioloog werkt nu als biodiversiteitinformaticus bij het Zoölogisch Museum in Amsterdam.

Er zijn aanwijzingen dat de sneeuwval is toegenomen in het oosten van Antarctica, waar Ardery ligt. Australische klimatologen beschreven in februari in het wetenschappelijke tijdschrift Nature Geoscience dat de aanhoudende droogte in West-Australië van de laatste decennia samenhangt met een toegenomen sneeuwval in het Antarctische gebied.

Creuwels wil graag terug naar Ardery om de situatie te bekijken. „Onderzoek aan Zuidpoolklieren op het continent vanuit Nederland bestaat niet meer. Ik moet in het buitenland solliciteren."

MarBEE in the news

Newly minted PhD Jeroen Creuwels was featured in NRC Handelsblad (7 dec 10). Increasing snowfall in the Antarctic is affecting brood survival of stormvogels who normally nest on steep rocky slopes where predators find it hard to maneuver. With increased snowfall, the predatory birds are able to gain access to these areas. Juvenile mortality is typically only a few percent but with the additional predation pressure, the losses were 20%. Increased snowfall in the "Antarctic desert" is related to climate change.

Dissertations granted by CEES in 2010

M. te Beest	The ideal weed? Understanding the invasion of *Chromolaena odorata* in a South African savanna Promotor Prof.dr. H. Olff, University of Groningen
F.G.H. Boersma	Mechanisms of bacterial selection in the mycosphere of Tricholomateous fungi Promotor Prof.dr.ir. J.D. van Elsas, University of Groningen
J.C.S. Creuwels	Breeding ecology of Antarctic petrels and southern fulmars in coastal Antarctica Promotor Prof.dr. W.J. Wolff , University of Groningen
D. Haydar	What is natural? The scale and consequences of marine bio invasions in the North Atlantic Ocean Promotor Prof.dr. W.J. Wolff, University of Groningen
G. Hopcraft	Ecological implications of food and predation risk for herbivores in the Serengeti Promotores Prof.dr. H. Olff, University of Groningen Prof.dr. A.R.E. Sinclair, University of British Columbia
P.M.G. Lourenço	Staging ecology of black-tailed godwits in Portuguese rice fields and correlations with breeding season events Promotor Prof.dr. T. Piersma, University of Groningen
S.P.M. Michler	Sex-specific strategies in a sex-biased world Promotores Prof.dr. J. Komdeur, Prof.dr.ir. J.M. Tinbergen, University of Groningen
U. Nunes da Rocha	Ecology of *Acidobacteria* and *Verrucomicrobia* in the plant-soil ecosystem Promotor Prof.dr ir. J.D. van Elsas, University of Groningen
J.J. Poos	Effort allocation of the Dutch beam trawl fleet Promotor Prof.dr. A.D. Rijnsdorp, University of Groningen
T.A. Revilla	Multispecies resource competition Promotor Prof.dr. F.J. Weissing, University of Groningen
L. Ross	Genetic conflict and sex allocation in scale insects Promotores Prof.dr. I. Pen, Prof.dr. L.W. Beukeboom, Prof.dr. F.J. Weissing, University of Groningen
J. Schröder	Individual fitness correlates in the black tailed godwit Promotor Prof.dr. T. Piersma, University of Groningen
C. Schmitz	The cost of living. Temperature compensation of the metabolic rate in plants Promotor Prof.dr. J.T.M. Elzenga, University of Groningen
P. Stevens	Adaptation of Ralstonia solanacearum biovar 2 to temperate climates Promotor Prof.dr. J.D. van Elsas, University of Groningen
C. Trierweiler	Travels to feed and food to breed. The annual cycle of a migratory raptor, Montagu's harrier, in a modern world Promotores Prof.dr.ir. J. Komdeur, Prof.dr. F. Bairlein, University of Groningen

Y.I. Verkuil The ephemeral shorebird: population history of ruffs
Promotores Prof.dr. T. Piersma, University of Groningen, Prof.dr. A.J. Baker, University of Toronto

Dissertations granted by CEES in 2010, prepared within another institution

P. van den Hout Struggle for safety. Adaptive responses of wintering waders to their avian predators
Promotor Prof.dr. T. Piersma, University of Groningen

K. Hutchings Parasite-mediated selection in an island endemic, the Seychelles warbler (Acrocephalus sechellensis). Promotores: Dr. D.S. Richardson, University of East Anglia & Prof. dr. J. Komdeur, Rijksuniversiteit Groningen.

C. Kraan Spatial ecology of intertidal macrobenthic fauna in changing Wadden Sea
Promotores Prof.dr. T. Piersma, University of Groningen, Prof.dr. J. van der Meer, NIOZ

M. Roodbergen Population dynamics of black-tailed Godwits in the light of heavy metal pollution
Promotor Prof.dr. T. Piersma, University of Groningen

CEES PhD Students in 2009

T. Aghajanzadeh *Razi Med. Diag. Lab Iran. / Ecophysiology of Plants*
Volatile sulfur compounds released by *Brassica* species as biofungicides in crop production

N.D.P. Bhola *UE-bursary / Community and Conservation Ecology*
Characterization of the scale of spatial heterogeneity in savanna systems as novel approach to improving conservation planning

A.I. Bijleveld *NIOZ / Animal Ecology*
Cost-benefit analyses of flocking in shorebirds, with a focus on information exchange

C. Burger *Animal Ecology*
Latitudinal dispersal as solution to insufficient adaptation to climate change

M. de Cássia Pereira e Silva *NWO / Microbial Ecology*
Assessment of the baseline of soil microbial functioning across a range of soils

R.F. Castillo Cajas *EU-Marie Curie / Theoretical Biology*
Modes of speciation and biodiversity patterns

V. Cordlandwehr *NWO-ALW / Community and Conservation Ecology*
Plant functional traits and assembly of plant metacommunities in fragmented landscapes

M.S. Cretoiu *Microbial Ecology*
Meta genomics-based exploration of soils and aquatic samples

J. van de Crommenacker
NWO-WOTRO / Animal Ecology
Stress and fitness in small bottlenecked populations of the tropical Seychelles warbler

W. Diao
Evolutionary Genetics
Genetics and genomics of presygotic isolation in *Nasonia*

S. Donadi
Marine Benthic Ecology and Evolution
Interactions between benthic communities and local resource conditions: bivalves, polychaetes and sediment stability

X. Du
Grant Chinese Academy of Sciences / Community and Conservation Ecology
Progress in neutral theory for ecological communities

A.L.F. Duarte
RUG bursary / Theoretical Biology
Division of labor in insect societies - integrating self-organization and evolutionary theory

S. Duijns
NIOZ / Animal Ecology
Foraging ecology of the Bar-tailed Godwit, *Limosa lapponica*

K. Elschot
OIO / NWO-ALW / Community and Conservation Ecology
Biotically created vegetation heterogeneity during early primary succession on salt marshes

F.A. Encinas Viso
UE-Bursary / Community and Conservation Ecology
Theoretical study of mutualistic interaction webs

J. de Fouw
NIOZ / Animal Ecology
Pervasive impact of intertidal migrant top predators on species diversity and size structure of its prey may lead to spatial knock-on effects between ecosystems

C. Fritz
RUG Bursary / Ecophysiology of Plants
Limits of Sphagnum bog growth: how can cushion plants outcompete *Sphagnum* species in Tierra del Fuego, Argentina

C.X. Garzon López
UE-bursary / Community and Conservation Ecology
Determinants and consequences of the spatial arrangement of trees in the tropical rainforest of Barro Colorado, Panama

M. van der Geest
NIOZ / NWO WOTRO / Animal Ecology
Fragile biodiversity linkages: production and consumption in a nutrient-poor seagrass-dominated intertidal ecosystem

S. Gerritsma
Evolutionary Genetics
Identifying genomic variationamong natural *Drosophila melanogaster* populations and its association to immunological resistance against parasitoids

E. Geuverink
Evolutionary Genetics
Genetics of sex determination in haplodiploid wasps

M.C.W.G. Giesbers
Evolutionary Genetics
Genetics of adaptation: mating behaviour and reproductive straties in *Nasonia* wasps

S. Grizard *Animal Ecology*
How microbes shape bird eggs

M. Hammers *ALW NWO / Animal Ecology*
Individual model based analysis of the causes of senescence in a wild living cooperative breeding vertrebrate species

P.R. Hardoim *UE bursary / Microbial Ecology*
Metagenomics of food crop endophytes

A. Hegemann *Vogelbescherming Nederland / Animal Ecology*
Adaptations to a changing environment: in search of bottlenecks in the annual cycle of skylarks

E.F. Hoogwout *NWO / Microbial Ecology*
Microbial diversity in potato

N.P.C. Horrocks *ALW VENI / Animal Ecology*
The role of seasonal dynamics in risk of disease and resource availability in life history evolution of larks in different environments

Ö. Inceoglü *NWO / Microbial Ecology*
Development of molecular method to assess microbial diversity in potato

A.B.F. Ivens *RUG bursary / Theoretical Biology*
Evolutionary ecology of the mutualism between yellow meadow ants and root aphids

K.M. Jalvingh *Evolutionary Genetics*
Genetic stress responses of *Drosophila* species in relation to their geographic distribution and population dynamics

T. Janzen The origin and maintenance of biodiversity

B. de Jong *Instituut voor Wetenschap en Techniek, België / University of Gent / Animal Ecology / Animal Behaviour*
Influence of testosterone on female life history traits and fitness in great and blue tits

E.M. Kanga *UE-bursary / Community and Conservation Ecology*
The consequences of land use change for biodiversity in Africa

R. Kentie *Ministerie van Landbouw, Natuur en Voedselkwaliteit / Animal Ecology*
Populatiedynamica van weidevogels

R. van Klink *Waddenfonds / VROM / Community and Conservation Ecology*
Biodiversity and nature management on mainland saltmarshes

T. Koevoets *Evolutionary Genetics*
The genetics of postzygotic isolation in the haplodiploid sister species complex of *Nasonia*

E. Koppenaal	*NAM / Community and Conservation Ecology* Effects of soil subsidence following gas extraction on Ameland related to autonomous dynamics in the Wadden Sea
A.L.W. Kuijper	*RUG bursary / Theoretical Biology* Genetic conflict and the evolutionary causes and consequences of sex determination systems
M. Langenhof	*UE bursary / Animal Ecology* Social Evolution in real time: the effect of frequency dependent selection on animal personlities
J. Leyrer	*NIOZ / MAVA Foundation / Animal Ecology* Survival repercussions of individual differences in the timing of a migration from West Africa to Central Siberia
T. Lok	*ALW NWO / Animal Ecology* Comparative demography of migratory and resident spoonbill populations with overlapping ranges
W.J. Ma	*Evolutionary Genetics* Ecology and mechanisms of sex determination in the asobara genus (hymenoptera: braconidae)
C.A. Mallon	*Microbial Ecology* Understanding the effects of bacterial diversity on community functioning and stability
F.S. Mandema	*Waddenfonds / VROM / Community and Conservation Ecology* Biodiversity and nature management on mainland saltmarshes
K. Meijer	*Evolutionary Genetics* Evolutionaire veranderingen in de biodiversiteit van Nederlandse insecten in respons op invasieve planten en insecten
L. Molleman	*UE bursary / Theoretical Biology* Human cooperation - the role of prosocial norms
R. Nazir	*HEC / NUFFIC bursary /Microbial Ecology* Potential mechanisms making bacteria fit in fungal- associated soil environmental niches
H. Ndithia	*Nuffic / Animal Ecology* Seasonal dynamics of reproductive strategies and disease in tropical grassland birds living in habitats highly threatened by land use change
I.A. Neven	*NWO-ALW / Ecophysiology of Plants* Southern Ocean primary productivity in a high-CO_2 world
S. Nolte	*Waddenfonds / VROM / Community and Conservation Ecology* Biodiversity and nature management on mainland saltmarshes
J.E. Oosten	*Rosalind Franklin bursary / Theoretical Biology* Social context and behavioural syndromes: models and experiments in fish

A. Ordoñez-Gloria *UE-bursary / Community and Conservation Ecology*
Trait diversity: consequences for the sensitivity of ecosystems to global change phenomena

T. Oudman *NIOZ / Animal Ecology*
Pervasive impact of intertidal migrant top predators on species diversity and size structure of its prey may lead to spatial knock-on effects between ecosystems

J. Ouwehand *Animal Ecology*
Possibilities and constraints in life-cycle adaptation to climate change

S. Paolucci *Evolutionary Genetics*
The genetic basis of variation in reproductive diapause in *Nasonia* wasps

W. Patberg *NWO-ALW / Ecophysiology of Plants*
Contribution of buoyancy cells to the nutrient supply of Sphagnum species.

S. Perez Vila *RUG Bursary / Evolutionary Genetics*
Ecology and evolution of fly parasitoid communities

F. van der Plas *UE-Bursary / Community and Conservation Ecology*
Species assembly at different trophic levels over environmental gradients in a savanna ecosystem

M. Poortvliet *Marine Benthic Ecology and Evolution*
Population genetics and phylogeography of the Mobulid rays *Mobula japonica* and *Mobula munkiana* in the Gulf of California and the Pacific

P.E. Puentes Téllez *NWO / Microbial Ecology*
Adaptation of Fecoli to heterogeneous conditions

I. Puga-Gonzalez *RUG bursary / Theoretical Biology*
Self-organisation of social behaviour in primates

R. Radersma *NWO / VICI / Animal Ecology*
Influence of sex-specific competition and density on demography in the great tit

J.L. Ruifrok *SBB / Community and Conservation Ecology*
How herbivores create landscape mosaics of different plant functional types in temperate ecosystems

L. Salazar Jaramillo *Evolutionary Genetics*
Comparative genomics of parasitoid resistance in *Drosophila* spp.

S. Santos *NIOZ*
Physiological performance of the Peppery furrow shell *Scrobicularia plana* (da Costa 1778) along the European coast

L. Schmaltz *RuG / Provincie Friesland / Animal Ecology*
Comparative demography of Ruff and Golden Plover

M.J.J. Schrama *NWO Pionier bursary / Community and Conservation Ecology*
Food web assembly in a dynamic salt marsh

E. Schut *Animal Ecology*
Mhc based sperm competition in the Seychelles warbler and the blue tit

M. Shahbaz *NUFFIC bursary / Ecophysiology of Plants*
Whole plant regulation of sulfur and nitrogen metabolism in Brassica as affected by elevated atmospheric CO_2

K. Sieben *NWO-ALW / Marine Benthic Ecology and Evolution*
Marine trophic cascades - effects of fish diversity on coastal production

J. Spoelder *HLB Laboratory / Ecophysiology of Plants*
Endophytes in potato tubers and their relation with the host plant

E.E. van der Vaart *NWO-TopTalent / Theoretical Biology*
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O.H. Vedder *EC / Animal Ecology*
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The interplay between speciation, dispersal and adaptation to local conditions in a heterogeneous environment, and its effect on food web/community structure

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Genetic basis of sex determination in the haplodiploid wasp *Nasonia vitripennis*

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Natural and anthropogenic impact on North Sea gelatinous zooplankton population dynamics: implications for ecosystem structure and functioning

J. van der Woude *ALW VICI / Animal Ecology*
Fitness consequences of intra-specific competition and social stress in the Seychelles warbler

H.-Y. Yang *Beijing Normal University / NIOZ / Animal Ecology*
Staging ecology of red knots in Bohai Bay, China

F. Zaki Mostafa Ali *Bursary Egyptian Government / Ecophysiology of Plants*
Salt tolerance in maize

M. Zhang *Microbial Ecology*
Bacterial-fungal interactions in soil and the role of plasmids

PhD projects - theses in preparation

Y. Afeworki
WOTRO / Marine Biology (Ocean Ecosystems)
Seasonal dynamics in the trophic ecology and energetics of an important grazer (the parrot fish *Scarus ferrugineus*) on the coral reefs of Eritrea in the Southern Red Sea

A. Ayrinhac
IOP Genomics / Evolutionary Genetics
Evolution of ageing

C.J. Camphuysen
NIOZ / Animal Ecology
Population Biology of lesser black-backed gulls in The Netherlands

S. Ferber
Marine Biology (MarBEE)
Mechanisms underlying the genetic structure of seagrass meadows: historical stochastic events versus adaptation

E.O. Folmer
Animal Ecology / Community and Conservation Ecology
Patchiness of food and the structure of shorebird communities

C.G. Gosling
NWO-ALW Pionier bursary / Community and Conservation Ecology
Biotic determinants of functional heterogeneity in a tropical savanna

S.M.A.C. van Heuven
EU Carbo Ocean / Marine Biology
Meridional carbon transport in the North Atlantic Ocean

M. Hinsch
ALW VICI / Theoretical Biology / Animal Ecology
Evolution of sex-specific reproductive tactics under density-dependent selection

J. Jansen
NIOO-CEME / Marine Benthic Ecology and Evolution
Exploring ecotypic variation in European shellfish populations

D.A.P. Reid
Rosalind Franklin bursary / Theoretical Biology
Self-organisation of fish schools

J. Reszka
UE Bursary / Evolutionary Genetics
Genetic basis of inbreeding depression in two insect species

M. van Rijswijk
Evolutionary Genetics
Dispersal and local adaptation in experimental metapopulations of *Drosophila melanogaster*

M.W. Smith-Kleefsman
Evolutionary Genetics
Determinants of metapopulation dynamics: stochastic processes versus adaptation

R.M. Veeneklaas
Community and Conservation Ecology / Population Genetics
Dispersal and adaptation in two salt-marsh plant species

E. van der Zee
NIOZ / Animal Ecology
A regime shift in bentic organisms in the Waddensea: causes and consequences for higher trophic levels

A.M. Zipperle

Ubbo Emmius Bursary / Marine Biology (MarBEE)
Inter-relationships between reproductive ecology, genetic structure and fitness in populations of the seagrass *Zostera noltii* in the Wadden Sea – North Sea

Animal Ecology

http://www.rug.nl/biologie/onderzoek/onderzoekgroepen/dieroecologie/index

Group leader Prof. dr. Theunis Piersma

Composition of the group in 2010:

Tenured Staff	**source**	**fte**	**period**
Dr M.W. Dietz	RUG	0.6	
Prof. dr. Ir. J. Komdeur	RUG	1.0	
Prof. dr. T. Piersma	RUG	0.7	
Dr. B.I. Tieleman	Rosalind Franklin Fellowship	1.0	
Prof. dr. J.M. Tinbergen	RUG	1.0	
Post-docs			
Dr. C. Both	NWO-VIDI	1.0	070101-120101
Dr. H.L. Dugdale	NWO-Rubicon	1.0	091101-101130
Dr. I.K. Lesna	PVE	0.3	080218-100815
Dr. K.D. Matson	NWO-VENI	1.0	081001-110930
Dr. J.W.H. Reneerkens	NWO-ALW	1.0	070201-100430
PhD Students			
Drs. C. Burger	RUG-FWN	1.0	080501-120430
Drs. J. van de Crommenacker	WOTRO	1.0	060101-100101
Drs. E.O. Folmer	RUG Breedtestr.3	0.8	031201-081201
Drs. S. Grizard	RUG/FWN	1.0	100101-131231
Drs. M. Hammers	NWO/ALW	1.0	081001-120930
Drs. A. Hegemann	Vogelbescherming-FWN	1.0	060301-101130
Drs. M. Hinsch	NWO-VICI	1.0	040901-080901
Drs. N.P.C. Horrocks	NWO-VENI/FWN	1.0	060401-100401
Drs. B. de Jong	IWT / RUG FWN	1.0	100101-121231
Drs. R. Kentie	LNV / RUG FWN	1.0	071216-111215
Drs. M. Langenhof	RUG-Ubbo Emmius	0.9	091201-140630
Drs. J. Leyrer	MAVA-NIOZ	1.0	050801-090801
Drs. T. Lok	ALW NWO	1.0	080301-120228
Drs. P.M.G. Lourenço	Portuguese Sci Foundation	1.0	060101-100101
Drs. S.P.M. Michler	NWO-VICI	1.0	040401-080401
Drs. J. Ouwehand	NOW/ALW	0.8	100513-150201
Drs. H. Ndithia	Nuffic	1.0	100601-140531
Drs. R. Radersma	NWO-VICI	1.0	070416-110415
Drs. L. Schmaltz	RUG-Prov. Friesland	1.0	091001-130930
Drs. J. Schroeder	RUG	1.0	040315-080715
Drs. E. Schut	RUG FWN	1.0	060901-100901
Drs. C. Trierweiler	St.Werkgr. Grauwe Kiekendief	1.0	050601-090601
Drs. O.H. Vedder	EC-FWN	1.0	061201-101201
Drs. Y.I. Verkuil	RUG	0.8	040315-090616
Drs. M.A. Versteegh	NWO-VENI/FWN	1.0	060401-100731
Drs. J. van der Woude	NWO-VICI	1.0	081001-120930

Technical/Administrative Staff	**source**	**fte**	**period**
secretary			
J.G. Rietveld	RUG	0.7	
Technical assistent			
Drs. J.C.E.W. Hooijmeijer	RUG	1.0	
Drs. R. Ubels	RUG	0.8	
Dr. M. van der Velde	NWO VICI	0.8	
IT-assistent			
Ing. G.J. van den Burg	RUG	0.2	
(part-time) field assistant			
R. Bijlsma	RUG	0.45	070101-111231
Dr. P.J. de Goeij	RUG		
R. van der Zee-Gietema	RUG		
Y.K. Galama	RUG		
Drs. N. Groen	RUG		
P. Wolfs	RUG		
R.P.W.M. Voesten	RUG		
H.M. Salomons	RUG		
A.D. Rippen	RUG		
Other PhD Students			
Drs. A.I. Bijleveld	NIOZ	1.0	090101-131231
Drs. S. Duijns	NIOZ	1.0	091001-130930
Drs. J. de Fouw	NIOZ	0.8	100501-150501
Drs. M. van der Geest	WOTRO-NIOZ	1.0	061001-101001
Drs. P.J. van den Hout	NIOZ	thesis defense in 2010	
Drs. K. Hutchings	NWO-VICI/U East Anglia	thesis defense in 2010	
Drs. C. Kraan	NIOZ	thesis defense in 2010	
Drs. M. Roodbergen	SOVON	thesis defense in 2010	
Drs. L. Walraven	NIOZ	1.0	100901-140901
Drs. E. van der Zee	NIOZ	1.0	090101-131231
Guests/Detachment			
Drs. R. van Dijk	University of Bath, UK		
Dr. P.J. de Goeij	Groningen,NL		
Drs. N. Groen	Groningen,NL		
Dr. P. Wiersma	Groningen,NL		
Yang Hong-Yan	East China Normal University, Beijing		

Introduction

The *Animal Ecology Group* is concerned with evolutionary, individual-based, explanations for the distribution and abundance patterns of animals as we encounter them in what is left of nature, and in the man-made landscapes that now dominate our planet. The search for factors moulding these distributions and abundances requires an appreciation of the selection pressures leading to adaptation and 'design' in individual animals under the pervasive influence of environmental factors. This leads to studies of life history evolution (including the historical constraints limiting possible evolutionarily stable solutions), and we try to reach an understanding by empirically emphasizing three 'trades' within the ecological sciences: (1) physiological ecology, (2) behavioural ecology and (3) population biology (Fig. 1).

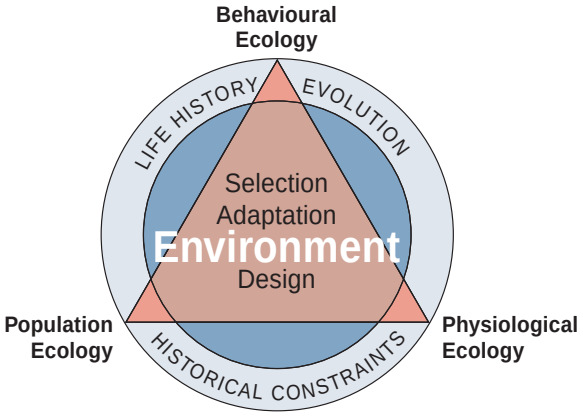

Figure 1. Diagram showing how members of the Animal Ecology Group think about their subject: explaining the distribution and abundance of animals from a deeply evolutionary viewpoint (with studies on selection, adaptation and design with due regard to the pervasive influence of environmental factors that has historically moulded life history evolution) and incorporating a variety of research approaches characteristic of different ecological 'trades'.

(1) *Physiological ecological* approaches help us delineate the mechanistic constraints limiting the option sets for different individuals and species. (2) *Behavioural ecology* puts individual animals squarely in environmental and social contexts, and examines the fitness consequences of alternative behavioural strategies. When possible, an experimental approach is chosen to determine the causality of relationships between behavioural variation and fitness, findings that we then try incorporate in our thinking about the population ecology of the animals under study. (3) *Population biology*, including demographic approaches, enable us to ascertain the consequences at the population level of environmental constraints on individual animals.

We try to keep an open mind to what new findings and developments in other fields of biological enquiry (such as theoretical biology, ethology, biophysics, genetics, endocrinology, immunology and molecular genetics) have to offer. Indeed, we particularly encourage the use of modern molecular techniques, both to determine genetic relationships between competing and cooperating individuals in a population and to decipher the long-term demographic and genetic histories of populations with a suite of consequences for today's functioning.

The demographic environment and the cost of reproduction

Richard Ubels, Rienk Fokkema & Joost M. Tinbergen

Reproductive patterns are shaped by natural selection. To learn more about this selection process, we have opted to measure fitness costs and benefits as a function of reproductive efforts. On the basis of our experimental fieldwork with great tits in the woodlands in the Lauwersmeer, we recently found that the demographic environment affected the cost of reproduction. Whether or not a parent with experimentally reduced reproductive effort survived better was dependent on the sex ratio of the offspring in its native area. In areas with an experimental male bias in the fledgling population, parents with reduced broods survived better than birds in the control and female-biased groups (M. Nicolaus *et al.* in review). This effect is important, because it can give us an evolutionary explanation for the negative effect of competition on reproduction.

We envisage a world where the genetic make-up of an individual, shaped by its ontogenetic environment, leads to a phenotype that in turn meets a selective environment that affects its fitness, fitness that feeds back into the frequency of genotypes in the population (Fig. 2, adapted from A.J. van Noordwijk). In practice the phenotype is not shaped exclusively on the basis of 'genetic instruction' during ontogeny, the phenotype is also affected by the environment, or even its own behaviour.

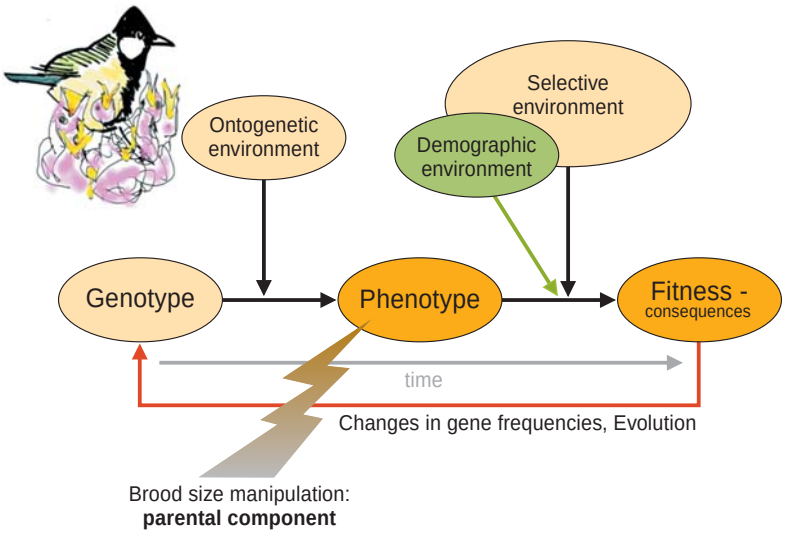

Figure 2. Scheme of effects of brood size manipulation on the parental component.

Our research on the fitness costs and benefits of reproduction have studied exactly this process. How does an increase in family size affect parental fitness output (Fig. 2, orange arrows)? We thus have experimental evidence that the demographic environment plays an important role in modulating parental fitness consequences of brood size and therefore is expected to affect natural selection.

Figure 3. One of our birds (female orange black/yellow aluminium) eating a berry of the sea-buckthorn (*Hippophae rhamnoides*). Although this is mainly a winter food source they can be of importance until the end of March.

We would like to know how this effect comes about. The hypothesis is that the resource-holding potential of the birds is affected by family size. This could cause a pattern in which the cost of reproduction only shows up when the demographic environment is such that it causes competition. We know that the brood size-dependent mortality takes place after midwinter, during a time that competition for food or other resources as sleeping places is most likely to be high. This competition could also be at play in early spring, when during the establishment of territories and/or nest boxes a revival of territorial behaviour takes place. To learn more about this we: (1) studied the spatial distribution of the birds in spring in relation to their previous experimental treatment (reduced control or enlarged brood) and (2) experimentally changed the nest box quality in spring to see whether birds with a different experimental treatment in the previous year do differ in their ability to occupy a high quality nestbox.

To study if resource holding potential is affected by reproductive effort, we manipulated reproductive effort of parents in the breeding season of 2009 by altering their brood size (reduced/control/enlarged) at day 6. All birds were individually marked with colour-rings (Fig. 3).

In the spring of 2010 we studied spatial behaviour of parents that raised a manipulated brood. We did so by weekly observations from the beginning of March until the beginning of May. During observations we registered the identity of a bird and the coordinates of their location (Fig. 4).

We related the distance between the breeding location in 2009 and the observations in the spring of 2010 to the brood size manipulation in 2009. In line with our expectation we found that parents with reduced reproductive effort were found closer to their breeding box in the year after (mixed model with variance at nestbox and individual level, controlled for observation week ($p < 0.001$) and sex (NS), $Chi^2 = 10.954$, df = 1, $p < 0.001$, Fig. 5).

Our results show that the spring habitat use of the parents was related to the manipulation of their brood size in the previous year. Parents with reduced broods were seen closer to their original breeding box as compared to parents with increased broods. This is consis-

Figure 4. Distribution of adult great tits with a 2009 manipulation history in spring 2010 in the Vlinderbalgbos (Lauwersmeer).

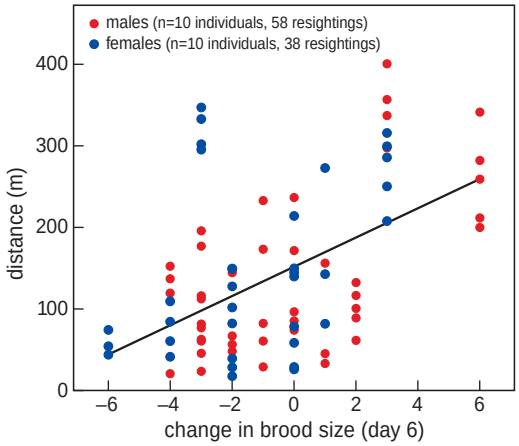

Figure 5. The relationship between the distance between the breeding box in 2009 and the observed location in spring 2010 as a function of the brood size manipulation.

tent with the hypothesis of M. Nicolaus *et al.* (in review) that reproductive effort affects competitive abilities. Depending on the environmental conditions (e.g. level of competition), this could translate in survival costs or loss of territoy (this study). Our results strongly suggest that the resource holding potential of the birds was affected by brood-size in the previous year and shows that this effect is likely to feed back into natural selection on reproductive efforts.

To test the idea that reproductive effort affects resource holding potential further, we changed the nest box quality in February 2011 by putting either no, one or two wooden blocs in the nestbox, reducing its depth (bottom to nest hole) from around 16 to respectively 11.5 and 7 cm. In studies by van Balen in the 1970s and 1980s it was strongly suggested that to a certain degree deeper nestboxes are preferred breeding places for tits. This preference may be related to the depredation probability. Breeding in shallow boxes may make the female, eggs and young more vulnerable for jays, woodpeckers or marten like predators that can grab their prey through the nest hole.

Such a preference for the deeper boxes is expected to cause competition between birds for the preferred nestboxes. With the

knowledge we have built up our expectation is that the probability to get a preferred nest-box should be related to last year's brood size treatment of the parents (Fig. 2). If parents with reduced broods are better competitors we expect them to be overrepresented in the preferred boxes. Also young may be affected by the size manipulation of the brood they were raised in.

Preliminary measurements of the choice of the breeding site show that the tits indeed have a preference for deep nest boxes (data from the very start of the breeding season April 2011, Fig. 6). Thus the first step of this experiment is successful: there is scope for competition for the deeper nest boxes!

We are now anxious to see whether last year brood size manipulation did affect the individual chances on a safe home! We expect that: (1) blue tits (the smaller species) will be outcompeted from the deep boxes by the great tits, and (2) that great tits with last years reduced brood sizes will be able to occupy the deeper boxes. We will report on this next year.

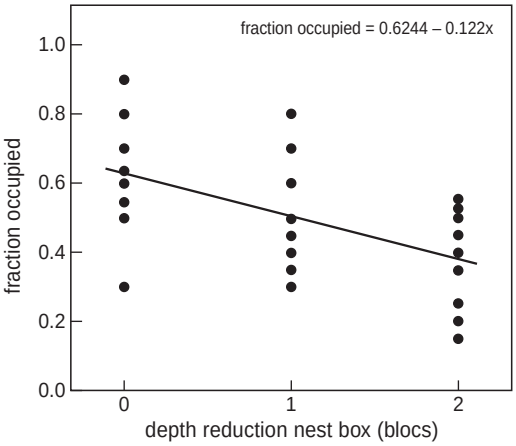

Figure 6. The relation between the fraction nest boxes occupied and the experimental reduction in nest box depth. Each dot represents a value for one of the twelve plots.

Experimental evidence for stabilizing selection on avian brood sex ratios

Reinder Radersma, Richard Ubels, Marco van der Velde, Joost M. Tinbergen & Jan Komdeur

Sex allocation theory is successful in predicting sex ratio variation in some taxa (e.g. haplo-diploid insects), but is generally a poor predictor of sex ratio variation in animals with chromosomal sex determination, such as birds and mammals. An important reason for this discrepancy may be that most models do not account for the complex life-history of birds and mammals, such as overlapping generations, multiple reproductive efforts, extended bi-parental care and sex-specific sibling interactions. To reveal the adaptive significance of sex allocation in birds and mammals, we conducted an experimental study on great tits in which we investigated the effect of manipulated brood sex ratios on long-term fitness benefits that accrue to both male and female parents.

We manipulated the brood sex ratios of in total 224 broods in 2008 and 2009. By exchanging 6-day-old nestlings of both sexes (Fig. 7) we created experimental brood sex ratios, uniformly distributed over the full range of brood sex ratios (0.0-1.0). In the subsequent breeding season we measured parental survival and fecundity (number of eggs produced in the first clutch) as well as offspring recruitment and fecundity. We related these fitness components to the brood sex ratio in the year before.

Figure 7. Great tit nestlings ready for transport to their foster broods.

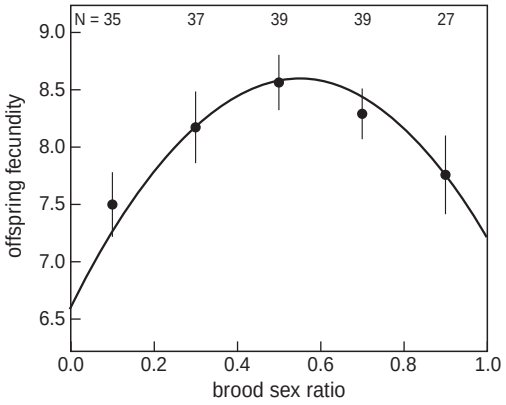

Figure 8. The effect of experimentally manipulated sex ratio of a brood an individual was raised in on its fecundity (number of eggs produced) in its first breeding season. Offspring raised in broods with a sex ratio at parity had a higher fecundity than offspring raised in broods with a sex ratio deviating from parity.

Offspring raised in broods with equal sex ratios had higher fecundity than offspring raised in broods with extreme biased sex ratios (Fig. 8). Since offspring recruitment, parental survival and parental future fecundity were not affected by the brood sex ratio these results mean that natural selection will favour brood sex ratios at parity (stabilizing selection).

We do not know how these differences in fecundity between individuals raised in a brood with a sex ratio at parity and individuals raised in brood with a sex ratio deviating from parity came about. Potentially the condition, competitive ability or social skills of the young were shaped by brood sex ratio and these differences were carried over until at least their first breeding attempt.

This study does however, shed light on an interesting evolutionary phenomenon. We argue that the stabilizing selection for equal brood sex ratios can provide an explanation why sex allocation in birds and mammals is often subtle. Higher benefits for equal brood sex ratios counteract selection for facultative sex allocation, when for instance interactions with relatives or environmental conditions favour brood sex ratios deviating from parity. We therefore hypothesise that stabilizing selection for equal brood sex ratios might help preserve chromosomal sex determination in birds and mammals. Over evolutionary time the mechanism of sex determination has shown to be a flexible trait in many taxa. As such, the maintenance of chromosomal sex determination is not trivial when there is no selective advantage for the outcome of this mechanism (brood sex ratios around parity). Stabilizing selection at parity provides this advantage and might be common in birds and mammals.

The thrills and challenges of migration ecology: a report from Ghana

Janne Ouwehand

The life cycles of migratory birds appear amazingly well adapted to temporarily exploit food peaks in breeding areas but avoid the harsh winter conditions by migrating to more hospitable environments. Being in the right place at the right time is of crucial importance to them. I study the potential for adaptation to changing climatic conditions on the breeding grounds in pied flycatchers, especially with respect to the constraints on the timing of migration. We examine the relative importance of genetic and environmental factors involved in mechanisms that can shift this timing, but also some of the potential ecological constraints further upstream that can hamper an adaptive response. This should provide better insight into how ecological conditions at the Sub-Saharan wintering grounds affect timing of migration, arrival and subsequent reproductive success of the birds. The wintering ecology of pied flycatchers is poorly studied and forms – relative to our well established nest box population with ditto personal accommodation in Drenthe – an incredible challenge. Information on how breeding and wintering sites are connected is missing.

So where to start, and how to study all this?... We opted for fieldwork in the middle of the wintering range in the politically stable and friendly country of Ghana, choosing two different study sites. The sites near the villages of Kintampo and Damongo are situated about 120 km apart on a latitudinal gradient. At these sites Christiaan Both, Claudia Burger and Rob Bijlsma encountered good numbers of wintering pied flycatchers during a pilot expedition in 2009. Together with a team of volunteers of ringers, (former) students, Rob Bijlsma and two staff members from the Centre for African Wetlands of the University of Ghana in Accra, we packed ourselves and piles of equipment into a 4x4 car to work in the field from early February to late April 2011.

After adjusting every method to measure changes in ecological conditions during the first month, we ran a pied flycatcher density count combined with an insect sampling

Figure 9. The early rains at the end of February changed the habitat in Kintampo rapidly from dry and open into lush and green in only three weeks time.

every two weeks. Insects of different layers were targeted using pitfalls, sweep nets and home-made flight interception traps that reached into the canopy. One would almost consider starting a bug collection, given the incredible variety that we saw in that class of animals! But we kept our focus on the birds. We tried to catch them, performed colour-ring reading, and observed (foraging) behaviour. In the heat of the day we spent our time sorting the insects to order level, and spent time on the toilet to beat a persistent type of diarrhoea. Pied flycatchers that left their droppings with us were also welcomed, as these faecal samples full of insect fragments helps us to understand the true diet. Together with foraging observations, insect sampling and habitat measures, we can then investigate relevant spatial and seasonal differences in food availability for the pied flycatchers. These seasonal changes from the dry to the wet season were very apparent in these wooded savannahs, especially in the most southern site Kintampo (see Fig. 9).

Despite the considerable efforts to set up a population of colour-ringed birds at both sites

Figure 10. This fully moulting Pied flycatcher male is deployed with a 0.5 gram geolocator developed by the Cornell University, Ithaca, U.S.A.

- to be able to site-fidelity and monitor individual departure dates - catching pied flycatchers remained very hard. Indeed, these birds tested our endurance to the utmost. Still, every one of the 62 colour ringed birds provided us with plenty of valuable data. Our ring readings indicated that at least part (but clearly not all) the birds that winter in Kintampo and Damongo are site-faithful. The 'best catch' by Rob confirms this even more. Before he even turned on the sound machine, 1 minute after opening the net, he recaptured a pied flycatcher ringed by Christiaan in 2009 (Damongo) only 70 m away from its original catching spot! This site-fidelity gives good hope for the 20 birds that we deployed with a geolocator (Fig. 10). Still, it will be hard work to find these birds with 'backpacks' full of data of migration routes and schedules back next year in one of our mistnets in Ghana!

The physiological measures that we took of each bird, provided information on migratory timing. The most striking is the enormous variation in individuals' body mass (Fig. 11), timing of moult and pre-migratory fattening (Fig. 12). Some seemed to be fully preparing for migration (18 grams, fat score of 5) in the beginning of April, while others were still busy moulting! That we did not resight all ringed pied flycatchers back, created ground for wonderful hypotheses. Are some of our captured birds just floating around without having a distinct wintering site? Are there different wintering strategies within this species: with both site-faithful birds as well as birds gradually moving northward to fatten along the way before a Sahara-crossing? Would such difference perhaps relate to breeding destination and the timing of spring there?

As pied flycatchers started arriving in The Netherlands in early April, in Ghana quite some birds remained. This makes one wonder about their breeding origin. I'm happy with

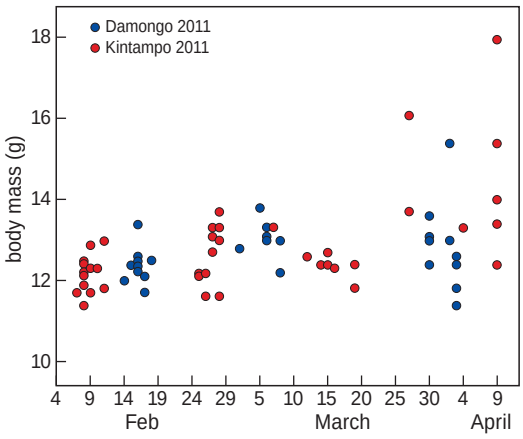

Figure 11. Only late in the wintering season birds start to increase their body mass.

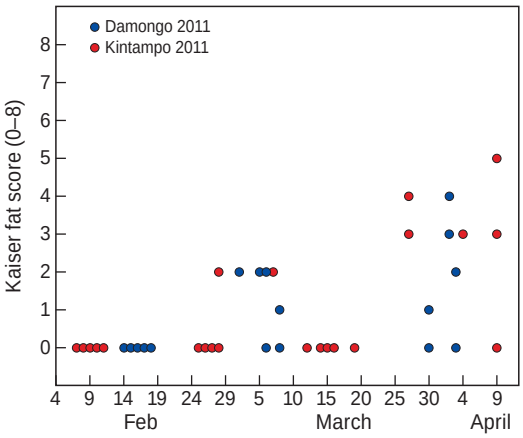

Figure 12. During the time that the first batch of pied flycatcher males had arrived in The Netherlands (early April 2011), the pied flycatchers wintering in Kintampo and Damongo just started storing fat as preparation for migration.

each tail and tertial feather that we collected from pied flycatchers in Africa. The feathers contain information on the location and quality of the breeding and wintering sites, respectively. Together with an enormous feather collection sent to us from all over the breeding range these can help us to describe migratory connections using stable isotope analysis.

We still wonder whether these wintering birds actually hold and defend territories. During February and March we hardly saw any territorial conflicts. In contrast to what is known, birds seem to tolerate each other for most of the time, even being just 50 cm away from each other. By the end of March we saw behavioural change, observing aggressive interactions between pied flycatchers. Not described so far were observations of pied flycatchers performing subsong and song. For the subsong they keep their beak closed, but you can see - from the throat shivering - and hear them (softly) singing. Occasionally they opened their beaks to make staccato, high frequency, tsii sounds. They seem to like combining bouts of subsong with foraging attempts.

Overall, this type of research requires staying sharp, daring to be flexible, adjusting to the conditions and expecting the unexpected! For me, this is a very satisfying experience of old-school-field-biology. I'm happy that I don't have to understand this complicated system in just one winter season. I hope that my upcoming analyses and next years' fieldwork will shed more light on some of these mysteries.

Age-dependent fitness components in the Seychelles warbler

Martijn Hammers, David Richardson, Terry Burke & Jan Komdeur

Individual fitness components, such as reproductive output and survival probability, often change markedly over time in many long-lived species. Understanding such changes is essential for understanding the evolution of life-histories. Generally, reproductive output and survival probabilities initially increase with age, before declining at old age. Although the processes of within-individual age-specific improvement are well understood, the study of late-life declines in performance remains contentious. The main reason for this is that, because of extrinsic mortality (e.g. predation, disease), only a very small number of individuals are present in the oldest age-classes. Possible explanations for late-life declines in reproductive output and survival probability include senescence (the deterioration of cellular and physiological function with age), and an increased risk for older individuals to contract a disease.

We used an exceptional long-term dataset of individuals from a natural, predation-free, closed and saturated population of the facultative cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*), where we could follow individually marked animals throughout their entire lives. We found that both reproductive output and survival probabilities show clear age-dependence, with an initial increase in relation to chronological age, and followed by a decline (Figs. 13 and 14), consistent with senescence. When we studied the age-dependent pattern in greater detail, we found that this pattern appears to be caused by age-dependence of the terminal reproductive attempt, where, compared to non-terminal breeding attempts, reproductive output was higher in mid-aged individuals, but lower in old individuals. In the years before the terminal reproductive attempt, reproductive output only showed weak age-dependence.

This age-dependence of the terminal breeding attempt may be explained by interactive effects of experience, physiological con-

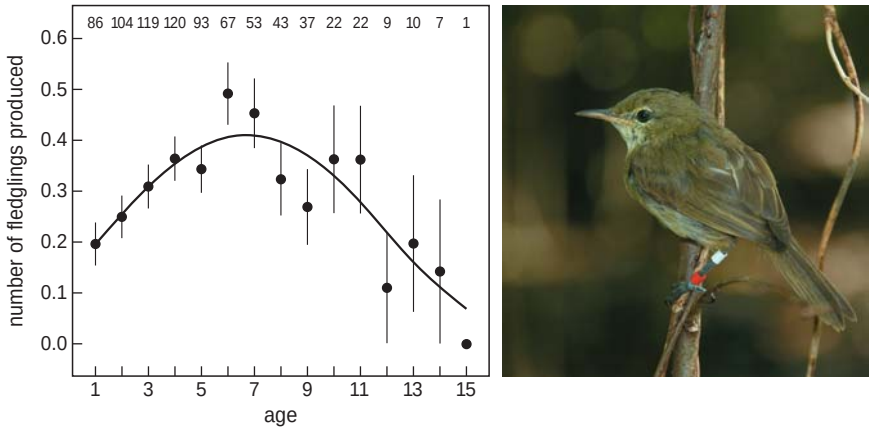

Figure 13. Reproductive output of female Seychelles warblers in relation to chronological age. Data are means and standard errors. Sample sizes are given for each age class. The solid black line shows the model predicted within-individual pattern of age-specific reproduction.

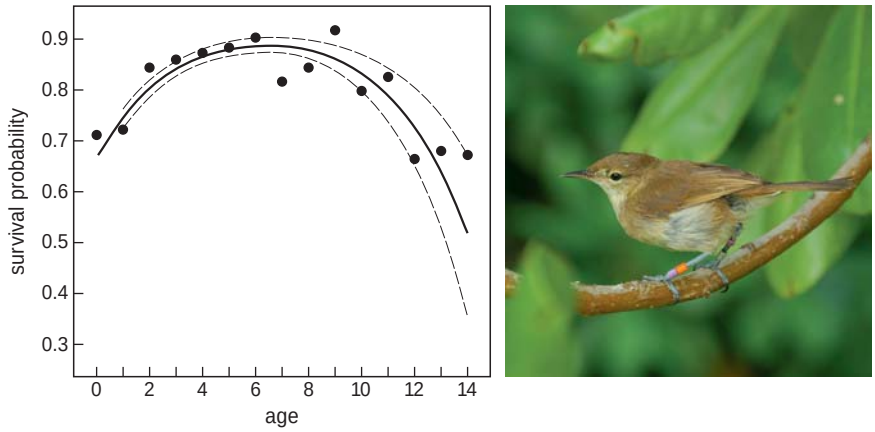

Figure 14. Annual survival probabilities in relation to chronological age in the Seychelles warbler. Data are average survival probabilities for each age class. The solid line is the model predicted regression slope for the model that was best supported by the data. The dotted lines are 95% confidence bands.

dition, terminal illness, and reproductive investment. These results indicate that, although reproductive output shows clear age-dependence, the underlying causes may be more complicated than previously thought. Terminal effects are often overlooked but may need to be considered to fully understand patterns of age-specific reproductive output in wild living animals. In future studies we will further investigate the causes of late-life declines in reproductive output and survival probabilities, for example in relation to physiological condition, social and environmental conditions, and different life-history strategies.

Fitness consequences of helping in the cooperatively breeding Seychelles warbler

Hannah Dugdale, David Richardson, Jan Komdeur & Terry Burke

Cooperative breeding is observed in birds, mammals, fish and insects. It is a social system in which group members care for offspring that are not their own. Individuals are selected to maximise their fitness, so why do some individuals help others to breed, rather than focusing on their own reproduction? Charles Darwin considered this apparently altruistic act to be one of the most important tests of evolution by natural selection. Kin selection theory later formulated how altruistic behaviour may evolve by natural selection; however, measuring fitness (the relative contribution of a genotype to the next generation) in natural systems remains problematic. One of the major questions in evolutionary biology is, therefore: why has cooperative breeding evolved?

Without cooperative breeding, competition for the limited breeding habitat will result in smaller territories and a pool of non-breeding individuals. Cooperative breeding may be the 'best of a bad job' – allowing otherwise unreproductive individuals to gain a small fitness benefit by helping to raise at least some additional relatives. Conversely, it may represent an alternative and equally productive life-history strategy if the fitness benefit of helping equals that of an unhelped breeder on a suboptimal territory. Comparing these alternative scenarios requires an understanding of how they affect lifetime reproductive success, in terms of the number of offspring raised in each generation. Furthermore, the recognition that helping has fitness benefits has led to the assumption that individual variation in helping is a plastic response to different environments. For cooperative breeding to evolve, however, there must be selection favouring helping, and helping must be heritable. It is vital to investigate the heritability of helping and its fitness consequences in order to gain a complete understanding of how and why cooperative breeding has evolved.

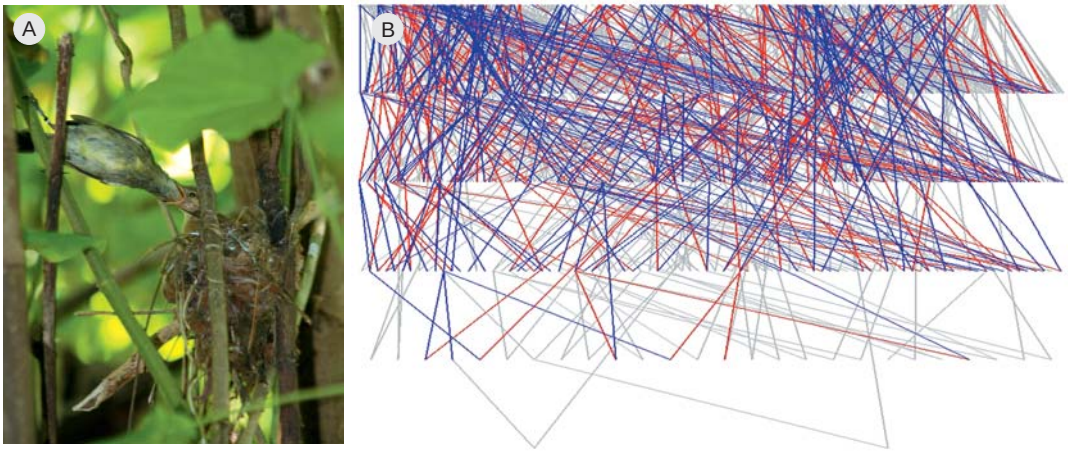

Figure 15. (A) a Seychelles warbler chick being fed at the nest (courtesy of Jildou van der Woude), and (B) the genetic pedigree of 1,057 warblers, split into six generations (the top-line is the first generation) with lines going from mothers (red) and fathers (blue) to offspring. Grey lines represent 544 birds that did not have helping data.

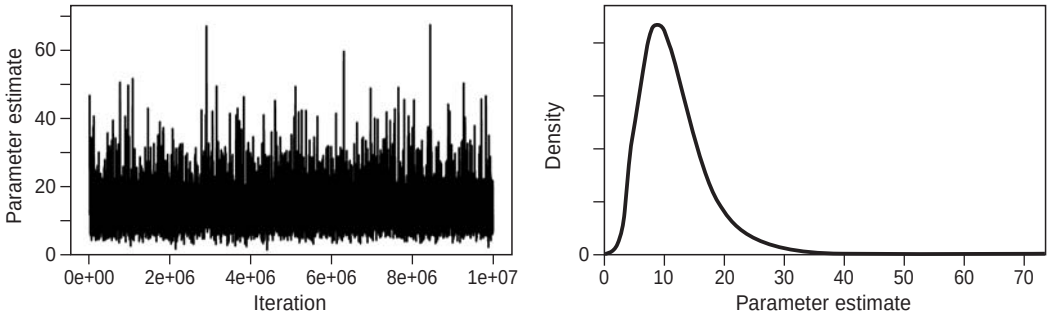

Figure 16. Markov Chain time-series (left) of 9,950 estimates of individual variation in helping. The posterior density (right) does not overlap zero, demonstrating individual variation in helping.

These questions have previously been intractable because they require powerful genetic markers (to accurately assign parentage), a closed natural population that is fully monitored (to accurately record survival, fecundity and life-history decisions) and long-term data (to monitor these parameters over complete lifetimes). The Seychelles warbler is a facultative cooperative breeder and is ideally suited for this purpose (Fig. 15A). This is because 30 microsatellite markers facilitate accurate parentage assignment (Fig. 15B) and there is virtually no immigration or emigration of Seychelles warblers between islands, enabling accurate measurement of both survival and fecundity. From 1997 onwards, nearly all of the warblers on Cousin Island have been individually colour-ringed, and their status (helper, non-helper, dominant) identified in each breeding season until death. It is therefore possible to follow the decisions of individuals over their lifetimes and to relate these to the realised fitness benefits. This is rare; hence, it offers one of the few cases where major evolutionary questions can be unravelled in a natural population.

Using Bayesian methods, we built a genetic pedigree of 1,057 warblers, of which 75 were and 438 were never observed helping. Warblers exhibited individual variation in helping behaviour (Fig. 16). Contrary to analysis of earlier years (1986–1995), the probability of helping was not sex biased, but it did show annual variation (1997–2006). A power analysis suggested that the pedigree had power of 0.8 to detect heritabilities greater than 0.24, but this did not enable separation of the individual variation into an additive genetic component.

Complete lifetime data were available for 209 warblers. Individuals that helped in their lifetime had greater lifetime fitness than those that did not, using both lifetime reproductive success (the number of chicks that a bird was assigned parentage of in its lifetime; $W = 6007$, $p < 0.001$) or individual fitness (λ, $U = 5579$, $p = 0.02$, Fig. 17A). De-lifed fitness (pt(i)) revealed that this was due to increased fecundity ($U = 5219$, $p = 0.04$), rather than increased survival ($U = 4909$, $p = 0.26$; Fig. 17B).

We therefore demonstrate selection for helping behaviour, and individual variation in this trait, which is a pre-requisite to heritability. A further 500 genotypes will be added to the pedigree. This will significantly and disproportionately increase the pedigree's power by increasing the mean depth to at least three generations. The current mean pedigree-depth is two generations; thus, adding 500 individuals will effectively double our power – a conservative analysis suggests that it will

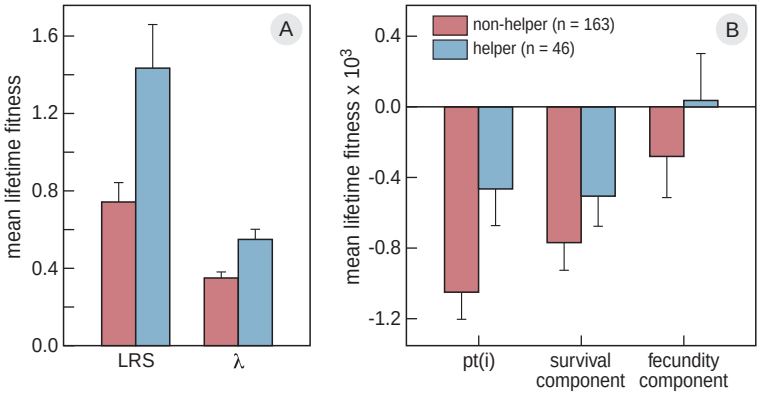

Figure 17. Mean lifetime fitness of Seychelles warblers that were observed helping or not-helping in their lifetime, where fitness was measured as A) lifetime reproductive success [LRS] and individual fitness [λ], and B) de-lifed fitness [pt(i)]. Error bars display the standard errors (S.E.); n = number of warblers.

provide power of 0.8 to detect heritabilities of at least 0.16.

Behavioural traits impact upon the fitness of individuals. Understanding the strength of selection on behaviour and the genetic and environmental components of these is vital for a complete understanding of evolutionary processes. Our findings improve our understanding of the adaptive basis of reproductive decisions in the cooperatively breeding Seychelles warbler, providing wider insight into the evolution of cooperative breeding.

Infection, immunity, and island-life

Kevin D. Matson, Sophie Vergouwen, Nicholas P.C. Horrocks & B. Irene Tieleman

Immune systems evolve in response to pathogens. Because of their isolation, oceanic islands are thought to have low pathogen pressures; therefore, island inhabitants might exhibit reduced immune function. In fact, some insular animals do exhibit increased disease susceptibility, and some insular populations declined spectacularly following pathogen introductions. To investigate how infectious threats shape immune systems, we study birds in the continental USA (Ohio) and on Bermuda, an isolated oceanic island. we quantify immunological and fitness (e.g., nestling growth, survival) parameters in the presence and absence of experimental treatments designed to alleviate disease pressure. We also collaborate with others on a parallel study investigating the infectious threats faced by eggs and the corresponding level of maternal investment in eggs and egg defenses. Overall, these integrative studies are designed to provide insight into how microbes, diseases, and avian immunological subsystems interact and vary across different locations and scales.

Islands, such as the Galápagos, are often seen as natural laboratories for studying evolution. One isolated island group that has received relatively little attention in terms of the evolution and ecology of its terrestrial

fauna is Bermuda. Located ~1000 km from the east coast of the United States, Bermuda (32°20'N, 64°45'W) comprises 138 islands totaling ~54 km^2. That means in terms of land area Bermuda is roughly halfway between Schiermonnikoog (~41 km^2) and Ameland (~60 km^2), yet more than 65,000 people reside there.

In 2010, we continued the broader line of research with a field trip to Bermuda. The Bermuda Institute of Ocean Sciences offered support and served as a research base there. Strictly speaking, the only oceanic element to my research is the vast distances of open water that isolate the birds that reside and breed on Bermuda from those in North America. But, it is precisely this isolation that allows us to ask questions about unique evolutionary trajectories associated with small populations that are naturally cut off from other populations.

Figure 18. Nest boxes in Bermuda (top) and Ohio (bottom).

Bermuda is a home to native eastern bluebirds (*Sialia sialis*) and introduced house sparrows (*Passer domesticus*), two species which also coexist in the eastern continental United States and which compete in both locations to use the same nest boxes. Despite the Bermuda's small size and high human population density, "bluebird trails" (managed nestbox networks) are maintained throughout the country (Fig. 18 top). Facilitating access to both species, these networks make Bermuda an ideal location to investigate questions about the physiological adaptations of land birds to isolated island environments. We compare the Bermudan bird populations with populations in the midwestern US (Kenyon College, Gambier, Ohio, 40°38'N, 82°40'W, Fig. 18 bottom), and these disjunct geographic distributions allows hypotheses to be tested within, rather than between, species.

We worked in Bermuda during May and June 2010 (Fig. 19 left). This period represents the first half of the breeding season and the period when most birds make their first, of what can be several, breeding attempts. We regularly checked 101 nest boxes spread over the eastern end of Bermuda. Bluebirds hatched at least one chick in 27 nest boxes. After establishing the hatch day (0), we visited bluebird chicks on days 2, 4, 8, and 15. The experimental treatment (lysozyme supplementation) or a control treatment (PBS) was applied on the first three visits, chick growth

Figure 19. Left. Sophie Vergouwen measures a bluebird nestling. Right. A 15 day old bluebird nestling weighs about the same as its parents.

was measured on every visit (Fig. 19 left and right), and two blood samples were collected one week apart on the last two visits. The experimental treatment was designed to boost immune function, since lysozyme is a naturally-produced antibiotic that chicks first absorb from the albumen and later generate endogenously. Additionally, we quantified the food delivery rate and subsequently caught, measured, and sampled the parents on days 12/13. Sparrows laid one or more eggs in 21 nest boxes, and overall we collected and analyzed 97 eggs. Specifically, egg sizes and masses were recorded, and the albumen (egg white)

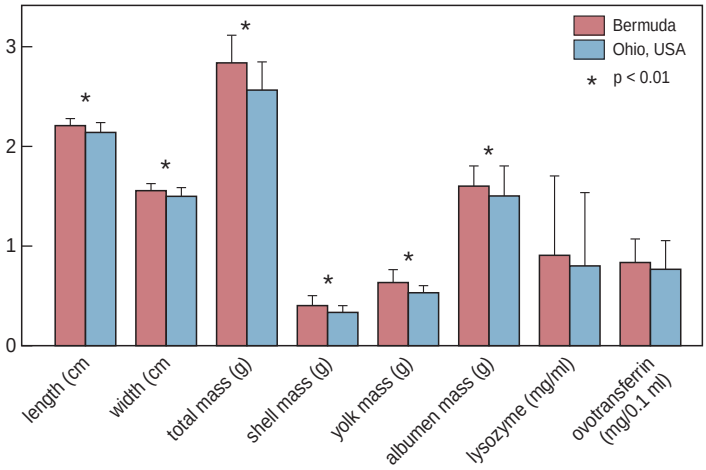

Figure 20. Comparison of house sparrow eggs from Bermuda and Ohio.

was assayed for antibacterial proteins that play a role in protecting an embryo from infection.

The most exciting results are those related to the comparisons between the locations, but these results must be considered preliminary since we only have data from one Bermuda season and the lab work is not yet completed. However, a picture is beginning to emerge. Bluebird nestlings differ between locations in terms of several nestling growth variables (e.g. the overall growth rate and the size on day 15 of both tarsus and wing). Lysozyme treatment affects nestling growth (primarily of the wing), but this treatment affects differently chicks that are smaller and those that are larger on day 2. Lysozyme treatment appears to have similar effects in both locations, suggesting indirectly that disease pressure might not qualitatively differ between Ohio and Bermuda. Due in part to larger sample sizes, we have a slightly better handle on the situation with the sparrow eggs. Bermudan sparrow eggs (n = 97) are significantly larger (heavier, wider, longer, etc., Fig. 20) than eggs from Ohio (n = 114). In contrast to our predictions, the concentrations of two anti-bacterial proteins, though not significantly different, are slightly higher in eggs from Bermuda (Fig. 20). In sum, these initial results suggest this research is on track to challenge some commonly held assumptions about ecological differences between islands and continents.

Genetic estimates of effective population sizes confirm high reproductive variance in a lekking species

Yvonne I. Verkuil, Cédric Juillet, Jos C.E.W. Hooijmeijer, David B. Lank & Theunis Piersma

An unsolved issue in studies of mating systems is the extent to which seasonal or apparent skews in mating success ultimately produce comparable skews in life-time reproductive success (LRS). Variance in LRS is an important species characteristic as it reflects the 'opportunity for sexual selection' (I_s), although it has been suggested that variance in LRS will only result in sexual selection under scenarios with a high degree of mate monopolization. Researchers wishing to determine variance in LRS need to obtain paternity analyses for at least one generation, as behavioural observations may not be accurate. Consequently, empirical evidence for the assumed low variance in LRS in monogamous species or high variance in LRS in polygynous species is often lacking. Here we take a population genetic approach to estimate the magnitude of skew in LRS in an extreme case of polygyny, lek breeding.

Variance in mating success leads to a smaller effective population size (N_e) than random mating. Under random mating, the theoretical ratio in the biparental effective size (N_e) and the maternal effective size (N_{ef}) is $\sim$ 2:1. Under the scenario of a strong male skew in LRS, the lek model of Chesser and Baker predicts that, depending on dispersal rates, the ratio N_e:N_{ef} can be as low as 1:3 (Fig. 21). A sex-specific skew in mating would thus lead to differential effects on N_e in sex-linked versus biparentally inherited genetic markers. A comparison of N_{ef} estimated with genetic markers that are only transferred by females, in comparison with N_e estimated with

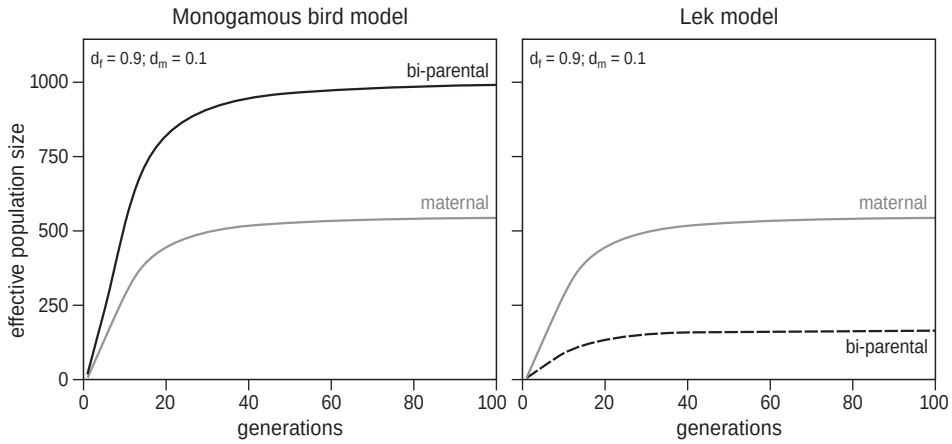

Figure 21. The Chesser and Baker (1996) predictions for biparental and maternal effective population size under two avian mating systems. Right: monogamous mating system, where male polygyny (Φ_m) is zero. Right: lek mating system, with high male polygyny (Φ_m = 0.9). In both models high female dispersal (d_f) and low male dispersal (d_m) is assumed, which is typical for birds. For explanation of models and model parameters see Chesser and Baker.

biparental markers, may help us establish any sex-related skews in LRS.

To assess whether the ratio N_e:N_{ef} deviates from the random mating model in a lekking species, we studied ruffs (*Philomachus pugnax*, Fig. 22). For ruffs is still unknown whether the strong seasonal skews in mating success between and within the permanent mating strategies of males, leads to unequally skewed LRS between the sexes. In females, variance in LRS is expected to resemble a negative exponential distribution as it largely is a

Figure 22. A ruff (right) courting a reeve (left) in April in our Frisian study area. Photo by Adri Hoogendijk.

function of survivorship, since clutch size is almost unimodal and double brooding is unlikely. The approach taken here is assessing effects on the effective size by measuring genetic variation in maternally and biparentally transmitted genes. The prediction is tested that the ratio N_e:N_{ef} is significantly lower than expected under random mating (2:1) and close to 1:3 as predicted by the lek model.

To obtain the effective population size from the genetic data the following equations need to be applied,

$$\theta_m = 2N_{ef}*\mu_m, \text{ for mtDNA,} \quad (1)$$

$$\theta_n = 4N_e*\mu_n, \text{ for nDNA,} \quad (2)$$

where θ_m and θ_n are the respective genetic variance parameters and μ_m and μ_n the marker-scaled mutation rates. (Note that the factor two in equation 1 accounts for the estimation of θ over two branches of the ancestral tree. The factor four in equation 2 accounts for the ploidy difference with mtDNA and the estimation over two branches). Estimates of the variance parameters θ_m and θ_n were obtained by coalescent analysis of mitochondrial haplotype data and microsatellite genotypes. Ruffs are not genetically structured across their range, so the haplotypes and genotypes were considered to come from a genetically uniform global population.

The random mating model of Chesser and Baker predicts a value of N_e:N_{ef} of approximately 2:1 (Fig. 21), however this model was not constructed for a case with unequal sex ratios. As adult sex ratio in ruffs is approximately 40% males to 60% females, the expected N_e:N_{ef} under a random mating scenario is (2*2/3):1, which is 4:3. Ratios significantly lower than 4:3 would indicate larger deviation from the random mating scenario in male than in female ruffs. The quantity N_e:N_{ef} is provided by equation 1 and 2,

$$N_e / N_{ef} = [\theta_n / (4 * \mu_n)] / [\theta_m / (2 * \mu_m)] \quad (3).$$

However, the uncertainty in μ_n and μ_m can largely affect the estimation of N_e and N_{ef} from genetic data. To include uncertainty in μ_n a range of 0.001-1×10^{-5} substitutions/locus/generation was assumed. For calculation of N_e a generation time of 3 years was assumed. For mtDNA, a μ_m of around 2–15% per Myr was assumed, which translates into 2×10^{-8} to 1.5×10^{-7} substitutions/locus/year (using control region sequence of 512 nt). This range includes published values for the mitochondrial genome of shorebirds.

For the estimation of N_e:N_{ef} , the Bayesian framework was used. To fully account for the uncertainty in N_e:N_{ef} , the widest range of the values of θ_n and θ_m and their 95% CIs as obtained from genetic data (see above) was used, and N_e:N_{ef} was estimated for all possible combinations of μ_n and μ_m. This means that for each given pair of mutation rates (μ_n,μ_m) the quantity N_e / N_{ef} was computed in presence of informative priors for θ_n and θ_m. The probabilities θ_n and θ_m were defined by two uniform distributions, U[6.160;8.650] and U[0.019;0.051], respectively, which means that all estimates within the range were weighed equally. To understand how the uncertainty in mutation rates affects the estimation of the quantity N_e:N_{ef}, the ratio is graphically represented as function of μ_n and μ_m.

The Chesser and Baker model assumes a high male polygyny (Φ_m = 0.9), but also high female and low male dispersal, which is a common feature in lekking species. In ruffs, high lek site fidelity has been observed in adult males. However, migratory males have shown large flexibility in migration routes, including breeding destinations, suggesting that dispersal may occur. Also, females might have lower dispersal than the model assumes. To test for sex-biased dispersal, the differentiation parameters as calculated from microsatellite genotypes were compared between males and females.

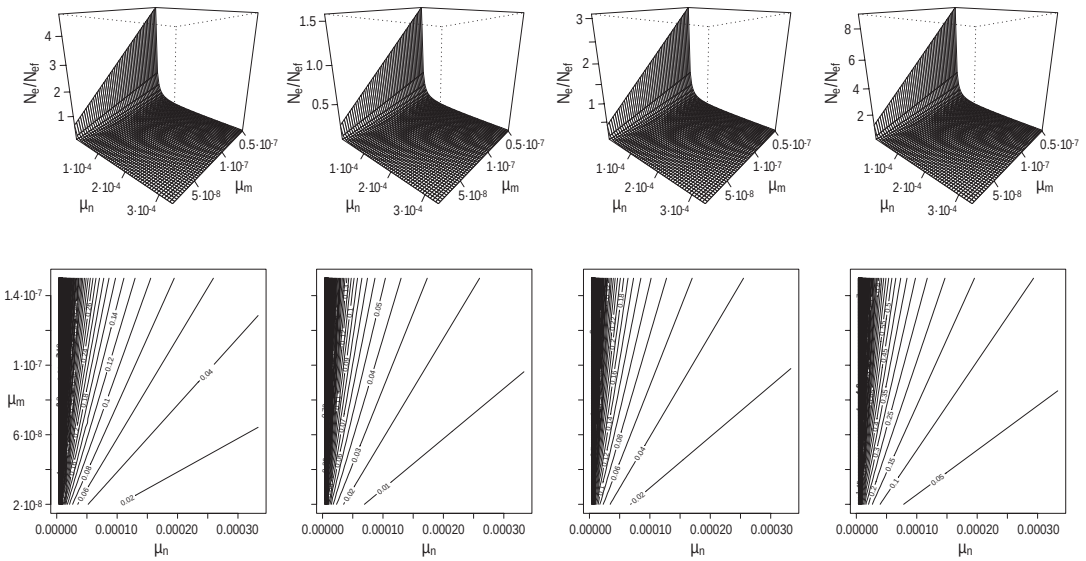

Figure 23. Distribution of the Bayesian estimate of N_e:N_{ef} and associated error (standard deviation, lower and upper limits of the 95% credible interval) as a function of a range of nuclear microsatellite and mitochondrial mutation rates (μ_n and μ_m, respectively). Isoclines on the lower graphs indicate values of N_e:N_{ef}. The nuclear population size N_e is defined by the relation $\theta_n = 4N_e{*}\mu_n$ and the mitochondrial effective population size Nef by $\theta_m = 2N_{ef}{*}\mu_m$. The probabilities of the genetic variance parameters θ_n and θ_m were obtained from coalescent analyses of genetic data.

Over a published range of mutation rates for avian control region sequence and vertebrate microsatellites and in presence of the uncertainty in the estimation of θ_n and θ_m, 92.2% of the distribution of N_e:N_{ef} was 1:3 or lower (Fig. 23), which is consistent with the lek model. Considering the distribution of the upper limit of the 95% credible intervals for Ne:Nef, less than 5% of the space covered included a ratio equal or higher then 1:1, depending on the assumption of a very low nuclear mutation rate ($\mu_n < 1\times10^{-5}$). Only 3.7% of distribution of the upper limit of the 95% credible intervals was equal to or higher than 4:3, the expected ratio for ruffs under a scenario of random mating, and climbing quickly over 8 for extremely low values of μ_n and high values of μ_m. Therefore given the estimates of θ_n and θ_m obtained in this study, random mating in ruffs was not supported

This is not the first study assessing effective population size in a lekking species, but it is the first to detect a sex difference in N_e. In the Gunnison sage-grouse (*Centrocercus minimus*), a ground-lekking bird, N_{ef} and N_{em} were similar because the variance in seasonal reproductive success was almost as high in females as in males, due to a high rate of nest failure. Likewise, in the lek-breeding European treefrog (*Hyla arborea*), no sex differences in effective size were found; a weak negative effect of the mating system was obscured by the much stronger effect of delayed maturity in both sexes. Our finding that N_e was lower than N_{ef} implies that male effective size (N_{em}) must be reduced in ruffs. Our results are consistent with the lek model of Chesser and Baker and implies that variance in LRS is larger in male ruffs than in females. However, variance in reproductive success is only one of the factors that affect long-term effective size as measured with genetic markers. Therefore other factors affecting N_e, other than variance in reproductive success, were

excluded: (1) unequal sex ratios were incorporated in the model, (2) in ruffs population fluctuations are stronger in females and hence could not explain the results, (3) age structure is similar in both sexes, (4) a significant Wahlund effect was found in females but not in males, which indicates higher dispersal tendencies in females than in males; this is consistent with the lek model, (5) selection could reduce genetic variation and although in this study the markers were not in significant linkage disequilibrium with each other, this does not exclude the possibility that one or more markers had lost variation due to genetic hitchhiking. Increasing the number of markers would reduce this problem.

Genetic evidence for strong reproductive variance in male ruffs presented in this study confirms the intuition of previous researchers who observed the large investment of some males in dominating the lek. Independent males may display so intensively that they refrain from eating and drinking, and lose mass throughout the breeding season. This study suggests that such investments indeed do pay off in relatively high LRS. Since females have extensive choice, their preferences largely determine effective population size in male ruffs. If female choice is directed towards 'good genes' (as in lek-breeding treefrogs) then directional selection could reduce genetic variation even further.

Reproductive success of black-tailed godwits in different kinds of meadowland

Roos Kentie, Jos Hooijmeijer, Niko Groen & Theunis Piersma

The Dutch black-tailed godwit population (*Limosa limosa limosa*) breeds on agricultural meadows. During the last decades, these meadows are more and more intensively managed to increase the yield of grass (Fig. 24 top). Intensification includes lowered groundwater levels, increased fertilization, reseeding of a protein rich grass species and the advancement of the first cut of the grass. Currently, meadows are being mowed during the nesting phase of the godwits. This results in an unfavourable breeding environment for the adults and an unfavorable foraging habitat for the adults and the precocial chicks, and it is the supposed cause of the severe decline of the godwit population in The Netherlands.

Extensively agricultural managed meadows (Fig 24 bottom) are often part of nature conservation organizations, and found in areas especially managed for meadowbirds. In these areas mowing is postponed until after their breeding period, groundwater levels are much higher, and the vegetation contains much more grass and other herb species. In this herb rich environment microhabitats are formed and more and larger insects, the prey items for the chicks, are found.

Although the intensively managed agricultural meadows seem to be detrimental for the breeding godwits, they still do breed here. Questions arise if this is a sustainable population, or if the intensive agricultural land functions as a potential ecological trap. Therefore we investigated the role of environmental conditions on the nests and chicks of Black-tailed Godwits breeding on extensive and intensive agricultural meadows.

Based on herb richness we classified the meadows in our study area in southwest Friesland as extensive or intensively agricul-

Figure 24. Different kinds of meadowland: intensive (top, mown at a time when black-tailed godwits still incubate eggs) and extensive (bottom; mown after the fledging of chicks).

tural managed (Fig. 25). From 2007 until 2010 we followed nests in the whole area, to measure nest success and to be able to mark the chicks when they just hatched. We weighed the chicks and put a metal ring with inscription and a flag with a unique code around their legs. The code flag enabled us to identify them with a telescope from a distance. We tried to recapture pre-fledging chicks of which we took their mass again. Based on resightings of marked chicks in subsequent years, we calculated their return rate.

In all years the nest success was lower on intensively managed meadows than on extensively managed meadows. In 2008 and 2009 more than twice as many nests hatched on extensively land compared to intensive managed meadows (Fig. 26). Although nests were often indicated with a stick, few nests on intensively managed agricultural meadows were destroyed by mowing activities. However, the lower nest success was mainly due to higher predation rates. Chicks that grew up on intensively managed meadows were lighter than chicks on extensive meadows (Fig. 27), but they did not differ in mass when they were born. That suggests that they faced poorer forage than chicks growing up on

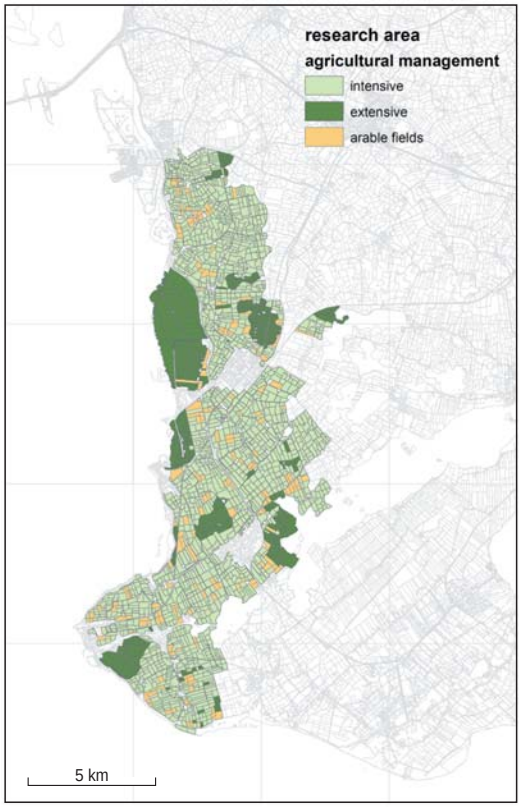

Figure 25. Mapping of different kinds of meadows in the southwest Friesian study area (2010).

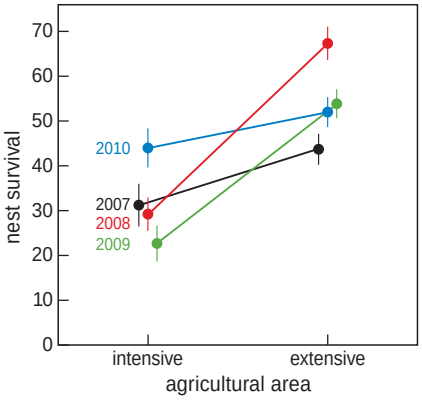

Figure 26. Nest survival of black-tailed godwits (calculated with correction for nest exposure, the Mayfield method) as a function of the kind of meadowland in 2007-2010 in southwest Friesland.

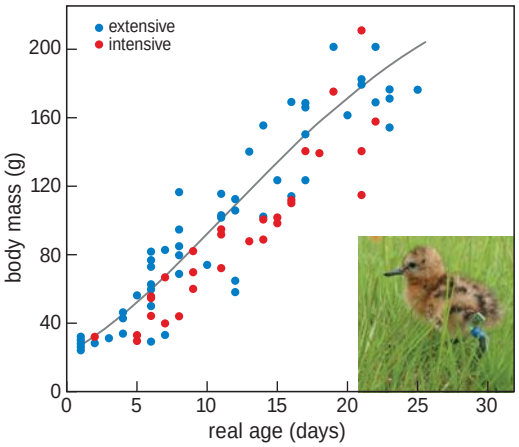

Figure 27. Growth of black-tailed godwit chicks as a function of the kind of meadowland in 2007–2010 in southwest Friesland. The Gompertz-curve is fitted for chicks born on extensive meadowland only.

extensive meadows. This effect may be even stronger than represented here, because we are unable to relocate chicks that might have died from starvation. Of the chicks that have hatched we resighted in subsequent years, five times as many chicks were born on extensive meadows (5%) than chicks born on intensively managed meadows (1%). The low food availability can cause this lower resighting probability, but it is also likely that chicks are victims of mowing machines. Moreover, because intensive managed meadows have less cover for chicks, chick predation can also play a crucial role.

The intensification of agricultural use of meadows has a strong negative effect on the reproduction of black-tailed godwits. Not only on direct output, but also on chick condition during growth. Low nutrition status in early life can affect further development and can have long term effects up into adulthood. For the conservation of this species it is very important to have enough extensively managed agricultural land, so that a high enough reproductive output can prevent black-tailed godwits from going extinct.

The functional response of bar-tailed godwits measured in the field: from individuals to populations

Sjoerd Duijns, Jan A. van Gils & Theunis Piersma

The bar-tailed godwit (*Limosa lapponica*) is a long-distance migrant of which two subspecies occur along the East-Atlantic flyway. These two subspecies follow a leap-frog migration, with the European Wadden Sea as staging and stopover site. The subspecies taymyrensis visits the Wadden Sea twice a year during one month to replenish stores needed for migration between breeding areas in northern Siberia and wintering sites in West Africa. This subspecies follows a time-minimizing migration strategy, faces a tight time schedule before leaving to the breeding grounds, and devotes as much time as possible for fuel deposition, while minimizing other energy consuming activities. By increasing foraging time, the time at the stopover site is minimized, and consequently time spent on migration is minimized. This study was performed during spring migration and focuses on the subspecies *taymyrensis*.

The functional response describes the relation between energy intake rate on the one hand and prey and competitor density on the other hand. The functional response can be considered the backbone of foraging ecology, yet it may be difficult to measure in the field due to rough and difficult conditions. Many ecologists have tried to use captive individuals of various species to measure the functional response and prey preferences for example. Bar-tailed godwit are difficult to keep in captivity and therefore the only way to study this species is out in the field. This is easier said than done since their is very diverse.

The study area was located at the tidal flats at the western part of the Dutch Wadden Sea, east of the island of Texel (Fig. 28). At various locations consisting out of different food availabilities 6×3 plots were erected from which food was sampled and observations were performed. At every location three

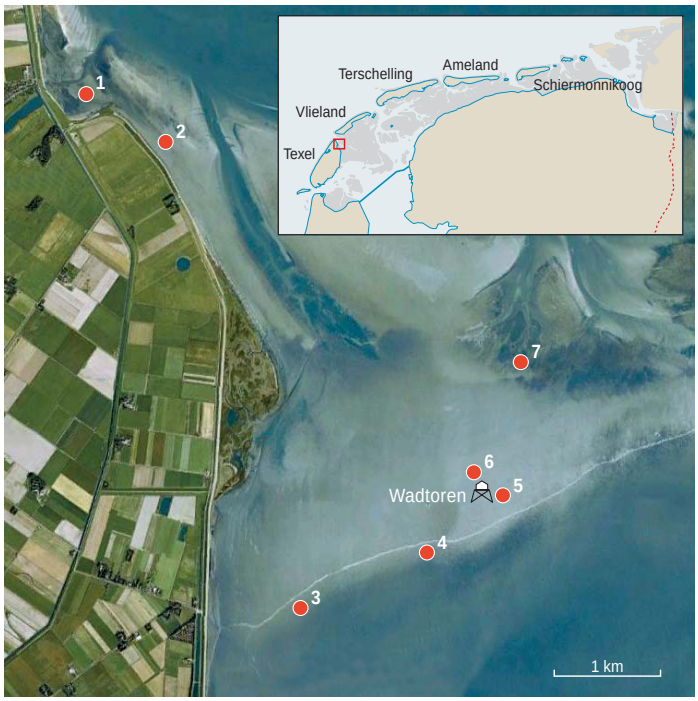

Figure 28. Overview of the study area with the location of the different areas (numbers)

Figure 29. Observing Bar-tailed Godwits in the different plots. Note the flock of foraging bar-tailed godwits and the poles of the different plots.

plots (50×50 m) were placed, 100 m apart. Observations (Fig. 29) were recorded on a digital voice recorder and analyzed after the field season. All prey items were scored and length was estimated based on bill length.

Halfway the study period, benthic samples were taken and stored in formalin, which were later analyzed and measured in the lab. We plotted individual intake rate on these estimates of food availability (Fig. 30). As bar-tailed godwits are highly dimorphic in bill and body size a distinction between the sexes was made. The curve of males has a good fit and seems to follow the Holling type-2 functional response, but the curve of females is different. This may be caused by females suffering from hidden interference competition, or because females forage near or in the waterline at the time when lugworms (*Arenicola marina*) are most active and close to the surface. In the case of females we may not have properly estimated prey availability.

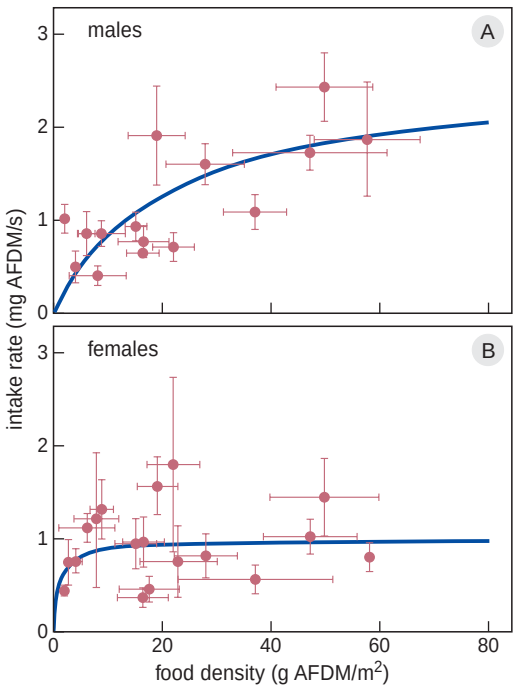

Figure 30. The relationship between the mean (± SE) intake rate (AFDM/s) and the mean (± SE) density of food density for male (A) and female (B) bar-tailed godwits. The function plotted is the Holling's disc equation $\left(\gamma = \frac{a * N}{1 + a * N * t}\right)$.

Parameter estimates are for males a = 0.123, t = 0.383 and R^2 = 0.205 and for females a = 0.85, t = 1.01 and R^2 = 0.018.

Spatial habitat modification by reef builders in an intertidal soft-bottom ecosystem affects several trophic levels

Els van der Zee, Tjisse van der Heide, Serena Donadi, Johan Eklöf, Britas Klemens Eriksson, Han Olff, Henk van der Veer & Theunis Piersma

In intertidal soft-bottom systems like the Wadden Sea, community structure can depend on the presence of species that can modify their environment. Such species, called "ecosystem engineers", strongly affect the physical space in which other species live, thereby affecting associated organisms. Reef builders such as blue mussels (*Mytilus edulis*) and Pacific oysters (*Crassostrea gigas*) are well known examples of ecosystem engineers. They provide structural protection and alter hydrodynamics by providing hard substrate and alter sediment conditions by accreting fine particles. Furthermore, they can create strong spatial gradients in these sediments conditions in the surrounding area through biodeposition of faeces and pseudo-faeces.

Macrozoobenthic biodiversity and biomass on mussel and oyster beds is usually higher compared to bare, sandy areas and both the associated fauna and the reef builders, form an important food source for many predators, such as crustaceans, fish and birds. Although the importance of mussel and oyster beds for the local benthic community and associated predators is well recognized, their importance to the surrounding area remains unquantified. By creating spatial gradients in sediment conditions, reef builders might not only influence trophic interactions at the local scale, but also at a large spatial scale.

We did an observational study on large-scale spatial engineering by reef builders in the Wadden Sea and documented their spatial effects on sediment conditions, macrozoobenthic invertebrate and shorebird distributions. We collected sediment and benthos samples on a predetermined grid (500m x 800m) at two

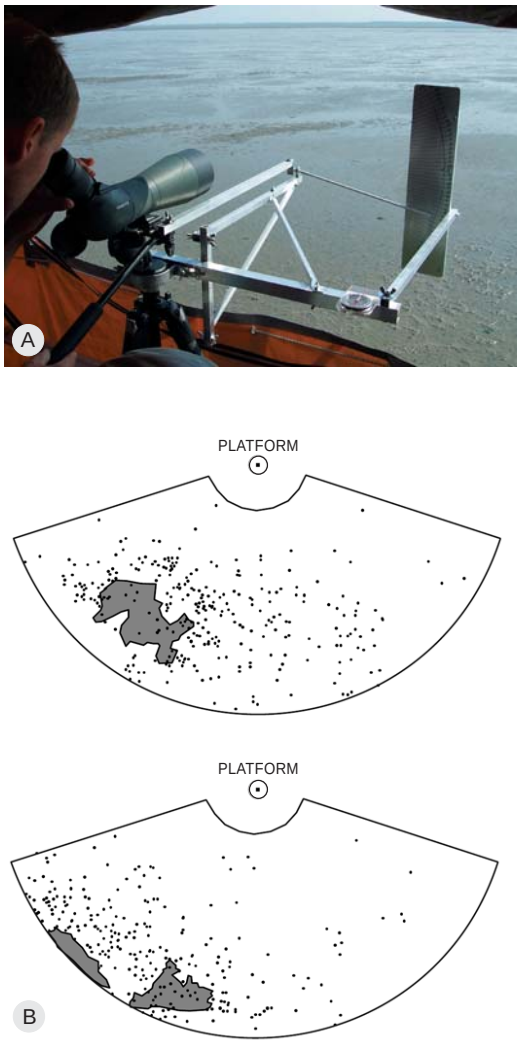

Figure 31. (A) The telescope mounted angulator and (B) an overview of both areas and the position of the reef builders beds in relation to the distribution of oystercatchers. Circles with black dot represent the positions of the two observation platforms. Black dots represent the positions of oystercatchers and grey polygons represent the reef builder beds.

sites on the Island of Schiermonnikoog. To determine the spatial distribution of 4 shorebirds we build a 3 m high observation platform in each study site and measured the positions of the shorebirds with the use of a telescope mounted angulator (Fig. 31). We applied path analysis to investigate direct and indirect interactions between the different components of the system.

We found a strong spatial gradient in sediment properties as a function of distance to the beds of mussels and oysters which indicates large-scale spatial engineering. The abundance of several benthic species correlated strongly with these sediment gradients, with higher biomass values close the mussel and oyster beds. Detailed measurements of the positions of 4 shorebird species demonstrated that reef builder beds establish food conditions for oystercatchers, curlews and bar-tailed godwits directly and indirectly through their effects on the surrounding sedimentary habitats (for oystercatchers and curlews, see Fig. 32). These shorebird species foraged more on and in the vicinity of the beds compared to the more sandy surroundings. Black-headed gulls were not correlated to the presence of a reef builder bed.

Our results show the importance of habitat modification by reef builders in a large-scale spatial setting. We underline the keystone function of ecosystem-engineers in soft-sediment ecosystems and emphasize that restoration of reef builders should be considered as an important step in the restoration of the Wadden Sea ecosystem.

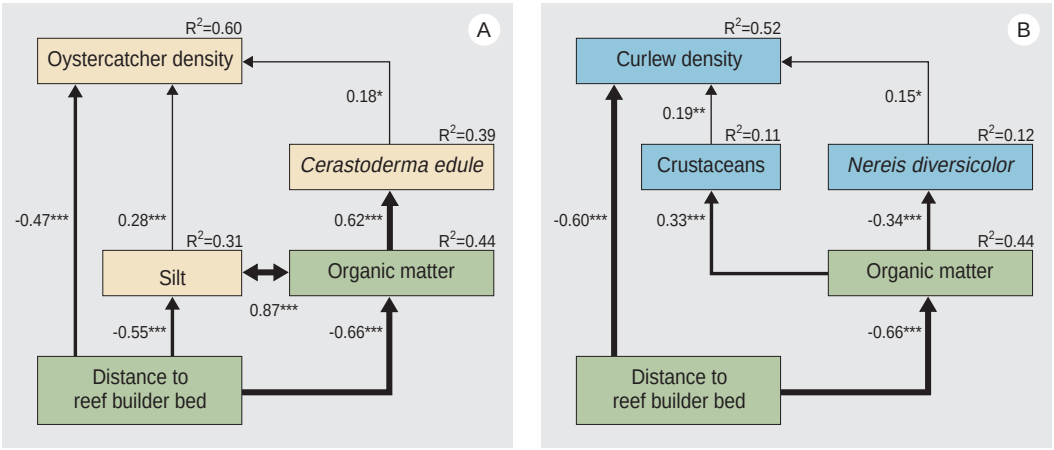

Figure 32. Diagram showing the final results of path analysis for (A) oystercatchers and (B) curlews. Straight arrows represent direct effect of one variable (boxes) on another. Double headed arrows indicate correlations between variables. The line size of an arrow indicates the magnitude of the standardized path coefficient, which is presented numerically next to each path.

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Doctorate granted by the institution, prepared within the institution

Lourenço, P.M. 2010. Staging ecology of black-tailed godwits in Portuguese rice fields and correlations with breeding season events. Promotor: Prof. dr. T. Piersma, Rijksuniversiteit Groningen

Michler, S.P.M. 2010. Sex-specific strategies in a sex-biased world. Promotores: Prof. dr. Ir. J. Komdeur, Prof. dr. J.M. Tinbergen, Rijksuniversiteit Groningen

Schroeder, J. 2010. Individual fitness correlates in the black-tailed Godwit. Promotor: Prof. dr. T. Piersma, Rijksuniversiteit Groningen

Verkuil, Y.I. 2010. The ephemeral shorebird. Population history of ruffs. Promotor: Prof. dr. T. Piersma, Rijksuniversiteit Groningen & Prof. dr. A.J. Baker, University of Toronto

Trierweiler, C. 2010. Travels to feed and food to breed. The annual cycle of a migratory raptor, Montagu's harrier, in a modern world. Promotores: Prof. dr. J. Komdeur, Rijksuniversiteit Groningen & Prof. dr. F. Bairlein, University of Oldenburg

Doctorate granted by the institution, prepared within another institution

Hutchings, K. 2010. Parasite-mediated selection in an island endemic, the Seychelles warbler (Acrocephalus sechellensis). Promotores: Dr. D.S. Richardson, University of East Anglia & Prof. dr. J. Komdeur, Rijksuniversiteit Groningen.

Kraan, C. 2010. Spatial ecology of intertidal macrobenthic fauna in a changing Wadden Sea. Promotores: Prof. dr. T. Piersma, Rijksuniversiteit Groningen & Prof. dr. J. van der Meer, VU

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Community and Conservation Ecology

Group leader Prof. dr. H. Olff

Composition of the group in 2010:

Tenured Staff	**source**	**fte**	**period**
Dr. R.S. Etienne	RUG/NWO-ALW Vidi	1.0	
Prof. dr. H. Olff	RUG	1.0	
Dr. Ir. C. Smit	RUG	1.0	
Post-docs			
Dr. D. Alonso-Gimenez	NWO/ALW Veni	1.0	071001-101001
Ir. T. van der Heide	NWO/ALW	1.0	090301-100301
Ir. T. van der Heide	Waddenfonds	1.0	100301-120301
Dr. E.J. Weerman	NWO/ALW	1.0	100515-120514
Dr. P.A. Jansen	NWO Veni	1.0	070501-100501
Dr. E.R. Chang	Canadian Research Council	1.0	080519-100601
Dr. Ir. E. van Leeuwen	NWO Rubicon	1.0	080701-100201
Project researchers			
PhD Students			
Drs. N.D.P. Bhola	Bursaal FWN	1.0	070901-110901
V. Cordlandwehr MSc	NWO/ALW	0.8	070601-110101
K. Elschot	OIO/NW)-ALW	1.0	081001-120930
F.A. Encinas Viso	Bursaal VIDI Etienne	1.0	081001-121001
Drs. C.X. Garzon-Lopez	RUG UE bursaal	1.0	060103-100301
E.M. Kanga MSc	Bursaal Nuffic	1.0	060101-100101
Ir. R. van Klink	Vrom Waddenfonds	1.0	090101-130101
E.C. Koppenaal	Bursaal NAM	1.0	081101-121101
F. Mandema	Vrom Waddenfonds	1.0	090101-130101
S. Nolte	Vrom Waddenfonds	1.0	090101-130101
Drs. A. Ordoñez-Gloria	Bursaal	1.0	070901-110901
Drs. A.L.D. van der Plas	Bursaal (Pionier)	1.0	090401-130401
J.L. Ruifrok	Bursaal SBB	1.0	081001-121001
Drs. M.J.J. Schrama	Bursaal (Pionier)	1.0	071115-111115
Drs. G.F. Veen	V.d.Leeuw/NWO (Pionier)	0.8	050401-100401
E. van Velzen	Bursaal	1.0	090101-130101
Drs. S.N. de Visser	RUG UE bursaal	1.0	061001-101001
X. Du	Chinese beurs	1.0	080901-120901
T. Janzen	Bursaal FWN	1.0	100906-140905
Project employee			
Research assistants and technicians in South Africa employed through the University of Kwazulu-Natal, Durban (UND) and in Tanzania			
R. Howison	UND/RUG	0.1	031101-110401
E. Mayemba	consultant	0.5	050101-

	source	fte	period
Technical/Administrative Staff			
secretary			
I.C. Jansen	RUG	0.5	
Chemical analist			
N.D. Eck	RUG	1.0	
botanical analist			
J.J. Hogendorf	RUG	1.0	
Y. de Vries	RUG	0.5	
IT-assistent			
Ing. G.J. van den Burg	RUG	0.8	
Guests			
Prof. dr. J.P. Bakker	Groningen, NL		
Prof. dr. J. van Andel	Groningen, NL		
prof. dr. M. Bertness	Brown University, USA		
prof. dr. E. van der Maarel	Groningen, NL		
prof. dr. K. Prach	Univ. South Bohemia, Czech Republic		
J.M. Drees	Groningen, NL		
R.M. Bekker	Groningen, NL		
P. Esselink	Groningen, NL		
J.Carnicer	Univ. Barcelona, Spain		

Introduction

The Community and Conservation Ecology group studies the interactions in ecological communities, to better understand the determinants of biodiversity, and to provide insight in how such communities can best be managed, restored and protected.

Species interact in ecosystems with other species and with abiotic factors through various types of interactions, often forming complex networks. Possible interactions include those between consumers and their resources, between organisms and abiotic (non-resource) conditions, between organisms and detritus compartments through excretion and mortality, non–trophic interactions between organisms (eg pollination or mutualism with microbes) and spatial interactions between neighbouring ecosystems through dispersal and nutrient flow. We therefore study organisms on different trophic levels (soil biota, plants, herbivores, predators) with emphasis on understanding the consequences of these interactions for the structure of ecological communities and the functioning of ecosystems. We do this by studying the key processes that determine the abundance and distribution of species in ecological communities, such as competition, facilitation, mutualism, plant-herbivore interactions and predator-prey interactions. In theoretical studies, we aim at unravelling the relative importance of stochastic and deterministic processes in structuring communities, and study the interplay of ecological and evolutionary processes.

We work both in the trophics and in the temperate zone to get a broad overview of the main processes that determine community structure and ecosystem functioning. In this, we try to combine theoretical, experimental and observational approaches. Our main study sites are located in the Netherlands (Wadden Sea, Oostvaardersplassen, Overijsselse Vecht), Tanzania (Serengeti) and South Africa (Hluwehluwe-iMfolozi). Particularly our research in the Wadden Sea area has significantly expanded since last year, with special focus on the role of ecosystem engineers (sea grasses, mussels, lugworms) on community and ecosystem dynamics.

At the end of 2010 Dr. ir. Christian Smit joined COCON as assistant professor on a tenure track position. His work focusses on positive species interactions and consequences for the dynamics and spatial organization of plant communities. A specific question addressed is how plant-plant interactions change along gradients consisting of both abiotic and biotic stress (incurred by consumers). This is studied at the salt marshes and coastal dunes at the Waddensea area (Schiermonnikoog) but also at (semi)arid steppes in Southern Spain. Furthermore, Chris is involved in various grazing studies in a wide range of ecosystems in the temperate zone – floodplain grasslands, heathlands, forests, coastal dunes and salt marshes – that all seek to understand the interplay of (large) herbivores with their environment.

The following sections contain specific areas of progress of our research during 2010.

Ecological Implications of food and predation risk for herbivores in the Serengeti

J. Grant C. Hopcraft

Large-scale animal migrations have profound implications for key ecosystem processes such as nutrient cycling, competition and predation between species, as well as socio-economic

Table 1. Landscape variables effecting the daily turn angles and step lengths of (A) migrating wildebeest and (B) migrating zebra in the Serengeti. (+ indicates that at least 75% or more of the posterior is greater than 0 for at least half of the animals. – indicates that at least 75% or less of the posterior is less than 0 for at least half of the animals. * indicates that at least 97.5% or more of the posterior is more than or less than 0 for at least half the animals. n/a indicates variables that were not included in the final model) ρ describes the concentration around the mean turn angle for the wrapped Cauchy distribution and μ describes the direction of the turn. α is the scale parameter for a Weibull distribution which defines the mean distance of the step length.

A)		Plains (n=10)			Woodlands (n=15)		
		Turn Angle		Step Length	Turn Angle		Step Length
		ρ	μ	α	ρ	μ	α
Food quality	Nitrogen	0	0	+	-	0	+
	$(Nitrogen)^2$	0	0	-	n/a	0	0
	NDVI	0	0	0	0	0	+*
	dNDVI	0	0	0	0	0	-
Food Abundance	Grass Biomass	0	0	0	-	0	-
	$(Grass\ Biomass)^2$	n/a	0	0	n/a	0	+
Predation	Woody Cover	0	0	0	-	0	0
	$(Woody\ Cover)^2$	n/a	0	0	n/a	0	0
	Water	-	0	+	-	0	-
	$(Water)^2$	n/a	0	-	n/a	0	n/a
Anthropogenic	Human	0	0	+	0	0	+
	$(Human)^2$	0	0	0	0	0	0
B)							
Food quality	Nitrogen	0	0	0	-	0	+
	$(Nitrogen)^2$	0	0	0	n/a	0	n/a
	NDVI	0	0	+*	0	0	+
	dNDVI	0	0	0	0	0	0
Food Abundance	Grass Biomass	-	0	+	-	0	-*
	$(Grass\ Biomass)^2$	0	0	-	-	0	+*
Predation	Woody Cover	-	0	-*	0	0	+
	$(Woody\ Cover)^2$	n/a	0	+*	0	0	n/a
	Water	-	0	+	-	0	-
	$(Water)^2$	n/a	0	0	n/a	0	0
Anthropogenic	Human	0	0	+	0	0	+
	$(Human)^2$	0	0	0	0	0	0

spin-offs for societies whose livelihoods are contingent on them. However, migrations are collapsing globally, which raises concerns about the persistence of these landscape-scale biological processes. This research investigates the distribution and movement patterns of wildebeest and zebra in the Serengeti (Figure 1). The wildebeest and zebra migrations in Serengeti are a major source of bushmeat for local people living around the park. In addition, the Serengeti attracts over 150,000 international visitors / year who come to see the mass migration of over 1.5 million wildebeest and zebra. Tourism alone generates over $740 million / year for Tanzania. The results of our research illustrate that wildebeest migrate so as to access the highest quality food available, whereas zebra migrate with wildebeest to decrease their exposure to predation (Table 1), which is a largely unexplored driver of migrations. Despite this, the single largest variable that determines the movement of both species is proximity to human disturbance. Wildebeest and zebra avoid areas with high disturbance and this is important for conservation for two reasons: (1) migrants might be forced to change their traditional routes because of shifts in global climate but human disturbance might block them from moving, which could lead to catastrophic declines in the future. (2) There are plans to build a national highway across the Serengeti, which would bisect the migration and fragment an otherwise contiguous ecosystem. If the highway continues, we should expect the movement patterns of both zebra and wildebeest to change in direct response to human conflicts, and this could have large implications for the stability of the Serengeti ecosystem which could lead to declines in tourism revenue. This research makes significant contributions to understanding these risks and outlines possible mitigation strategies.

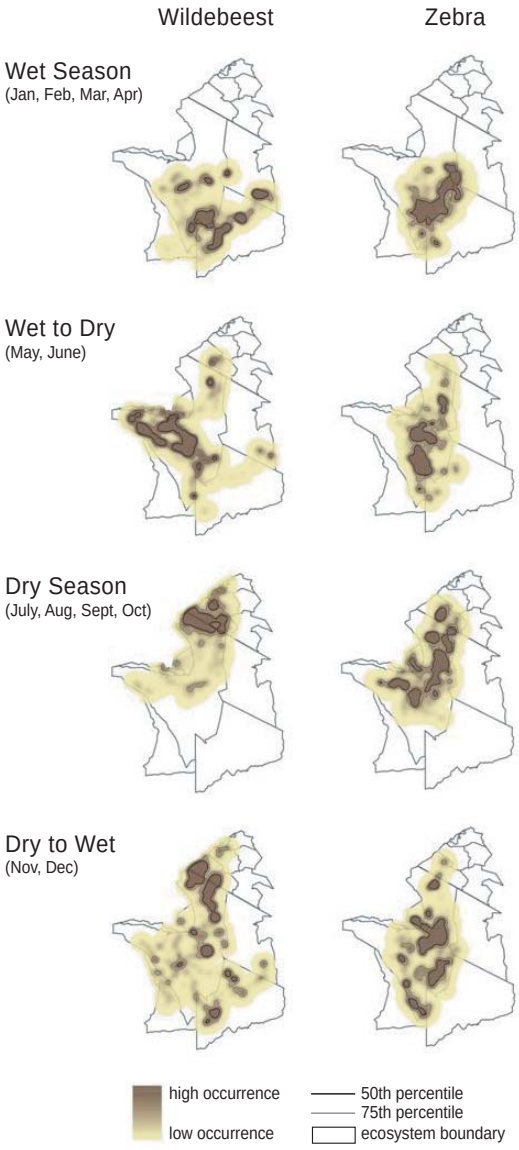

Figure 1. The seasonal distribution of radio collared wildebeest and zebra suggests that the zebra movement tends to be more diffuse than wildebeest. Wildebeest are constrained by food requirements and move in a coherent triangular pattern around the ecosystem. Zebra gain sufficient energy from lower quality grass provided there is sufficient biomass and move in less consistent linear north-south patterns.

Understanding plant invasions: A global scale meta-analysis

Alejandro Ordonez, Han Olff

The globalization of human activities has resulted in the intentional and un-intentional movement of animal and plant species to areas beyond their natural range. This has ultimately resulted in both the biotic homogenization of natural areas across the world and irreversible changes to the functioning of various ecosystems. Given that both of these changes pose a significant threats to indigenous flora and natural ecosystems, understanding the why, when and how of biological introductions is crucial. Because of this, one of the main goals of invasion biologist and community ecologists has been determining what makes a given species invasive or a community prone to invasions.

In an effort to reach this goal, the biological introductions problem has been addressed using two alternative perspectives: a species-based (asking, which species are invasive?); and a community-based (asking, which habitats are most likely to be invaded?). Each of these perspectives provides complementary answers to the "*what drives invasions*" question. Therefore, any progress towards a general theory of plant invasiveness can only been achieved by pooling evidence from both perspectives.

Several approaches have been used to answer these questions (e.g. population dynamics, invasive history, human use, trade intensity); but so far, the use of functional traits has proven to be one of the most promising one. This is due to the importance of certain traits (e.g. carbon capture, reproductive effort or light competition) in determining plant performance, and hence their success. In the case of invasion biology, describing the invasion process in terms of key attributes, and especially those related to performance and metabolic homeostasis, has allowed scientists to formulate some of the most accepted hypotheses explaining aliens success (e.g. evolution of competitive ability, novel weapons, new niches, Drawing's naturalization hypothesis). In these hypotheses, as in classical community ecology, the success of a colonizing alien is considered to be result of the influence on plant performance of environmental gradients (a species' expected performance is a function of both its fundamental niche and functional traits), and biotic interactions (changes in species optimal performance due to biotic interactions that shape a species fundamental niche).

Our work in this subject has addressed the invasiveness/invasibility question by exploring global patters of trait (dis)similarity between co-occurring alien and native plants. In order to do so, two questions were asked: Can trait (dis)similarity between aliens and natives explain non-natives success?; and, are the observed (dis)similarity patterns a product of evolution?. To answer these questions we first evaluated the relationship between native and alien plant performance related traits aiming to determine the existence of a pattern of trait (dis)similarity between both groups. Second, native-alien trait (dis)similarity patterns were evaluated in relation to the ecological setup (community phylogenetic composition, scale and resource availability) of the area where they are introduced. Lastly, the role of evolutionary dynamics as the mechanisms generating the observed patters of trait (dis)similarity was evaluated.

The first approach used by us in this work is to determine the traits of successful invaders. As prior studies have shown, the search of those attributes that allow a given

Table 2. Comparison of mean trait values of alien and native species, considering all species, and species grouped by growth form. Comparisons were made using linear mixed models; t and P values from these analyses are given in the right-hand column (significance levels indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, N.S. non–significant). Trait abbreviations: SLA – Specific Leaf Area (cm^2g^{-1}), Hmax – Maximum Canopy height (m) and SWT – Seed weight (mg). All traits were log10 transformed before these analyses. Additional information includes standard errors (SE) of trait means, the number of 25x25 km^2 communities involved in each comparison ("no. of Sites"), and the number of species in each group ("no. of species").

	Trait [no. of Sites]	Alien Mean (SE) [no. of species]	Native Mean (SE) [no. of species]	Linear mixed model
All	SLA [138]	133.3 (1.06) [788]	115.1 (1.04) [3164]	t = –5.51 ***
	Hmax [190]	3.3 (1.14) [647]	3.9 (1.11) [3562]	t = 3.71 ***
	SWT [190]	5.6 (1.19) [491]	7.6 (1.18) [2319]	t = 2.8 **
Graminoids	SLA [22]	155.7 (1.08) [39]	116.4 (1.09) [206]	t = –2.52 *
	Hmax [31]	0.6 (1.12) [42]	0.6 (1.14) [295]	t = –1.18 N.S.
	SWT [24]	0.4 (1.38) [31]	0.7 (1.26) [198]	t = 2.01 *
Herbs & forbs	SLA [57]	172.2 (1.09) [166]	130.5 (1.07) [610]	t = –3.99 ***
	Hmax [64]	0.3 (1.13) [172]	0.3 (1.09) [652]	t = –1.75 N.S.
	SWT [61]	0.7 (1.17) [155]	0.9 (1.17) [510]	t = 0.51 N.S.
Shrubs	SLA [36]	125.3 (1.1) [269]	114.2 (1.08) [386]	t = 1.36 N.S.
	Hmax [49]	1.5 (1.12) [106]	1.5 (1.1) [265]	t = –1.73 N.S.
	SWT [44]	1.7 (1.41) [67]	1.4 (1.4) [198]	t = –0.85 N.S.
Trees	SLA [76]	107.8 (1.09) [238]	101.8 (1.06) [1088]	t = –1.82 N.S.
	Hmax [109]	17.4 (1.08) [224]	15.5 (1.07) [1503]	t = 0.49 N.S.
	SWT [107]	31.7 (1.21) [176]	50.8 (1.18) [889]	t = 1.55 N.S.
Vines	SLA [11]	260 (1.19) [24]	243.6 (1.12) [74]	t = –1.43 N.S.
	Hmax [11]	3.3 (1.46) [25]	3.2 (1.38) [57]	t = 0.57 N.S.
	SWT [12]	39.2 (1.39) [21]	22.4 (1.55) [74]	t = –0.24 N.S.

species to be successful when introduced (species-based approach) has yielded some interesting results. Specifically, aliens have been found to have faster grow rates, higher leaf nutrients contents and specific leaf areas, shorter life cycles, devote more resources to reproduction and produce more seeds that are better dispersed and germinate faster (Table 2). Nonetheless, expressing any of these attributes would give an advantage only on particular situations (e.g. having a large leaf area does not have the same implication in a desert than in a tropical rain forest). Additionally, the biological context (traits of co-occurring species) also plays a major role on the advantages of particular traits.

In this work, both factors were considered by comparing aliens and native communities on particular locations. Evidence for inherently superiority of aliens when compared to co-occurring natives was observed. It is important to emphasise that, although the summarized patterns of differentiation (in uni- or multivariate trait spaces) seem rather small in absolute (15 to 26%) or relative terms (2 to 16%); It is very difficult to predict how big a trait difference should be in order to be of ecological relevance (e.g. in competition). These differences on traits representing an approximately independent axis of trait/strategy variation also hold for comparisons based on the multivariate trait composition (SLA-Hmax-SWT 3D or 2D spaces, Fig. 2) and their positioning along the *"leaf economics spectrum"* (Fig. 4). Analysis of the position of aliens in this multivariate trait-space revealed that non-natives clustered in trait values towards the edge of at least one of the evaluated dimension, when compared to natives Fig. 2). This suggests that the suite of traits expressed by aliens provides them with a competitive advantage from co-occurring natives (e.g. higher specific leaf areas, shorter life cycles, devotion of more resources to reproduction and production of more seeds that are better dispersed and germinate faster). Nonetheless, the large variability of alien-native differences across sampled locations indicates that site conditions ultimately determine the level of differentiation between these groups (as shown in Fig. 3, and by a significant effect of sites in trait comparisons). As a result any performance advantage of an alien with respect of co-occurring natives is site specific.

When the comparisons between aliens and natives were done within a similar growth form, functional group or between closely related species (experimental paring of species based on functional, phylogenetic or taxonomic similarity) traits of aliens were found to converge natives. This convergence (in a functional or phylogenetic space) seems counterintuitive when compared to the dissimilarity of aliens to most natives. A possible explanation for this is the balance between the availability of new niches to be invaded (intermediate positions between native species niches) and constraints imposed by functional similarity (intermediate positions become the worst places in the ''*fitness landscape*''). As a result of this balance, those areas between natives in a given area can only be invaded by highly competitive species); whereas the areas between functionally close species are relative windows of opportunity where even relatively weak aliens can be successful if they are functionally similar to those natives.

The second dimension evaluated in this project is the relation between community conditions and alien success. Several hypotheses aim to determine how community factors relate to the likelihood of at location being invaded (e.g. biotic resistance hypothesis, fluctuating resources, and the resource-enemy release hypothesis). In this work two of these alternative hypotheses, linking com-

munity properties to alien success, were evaluated within the framework of ecological traits: the biotic resistance and the resource-enemy release hypothesis. Both of these hypotheses can be related to the idea of "resource opportunities" (i.e. the net result of the effects of all the organisms in a system and the supply of the resource) and how these define the conditions promoting invasions.

The biotic resistance hypothesis is one of the oldest principles in invasion ecology, and is based on the idea that communities with

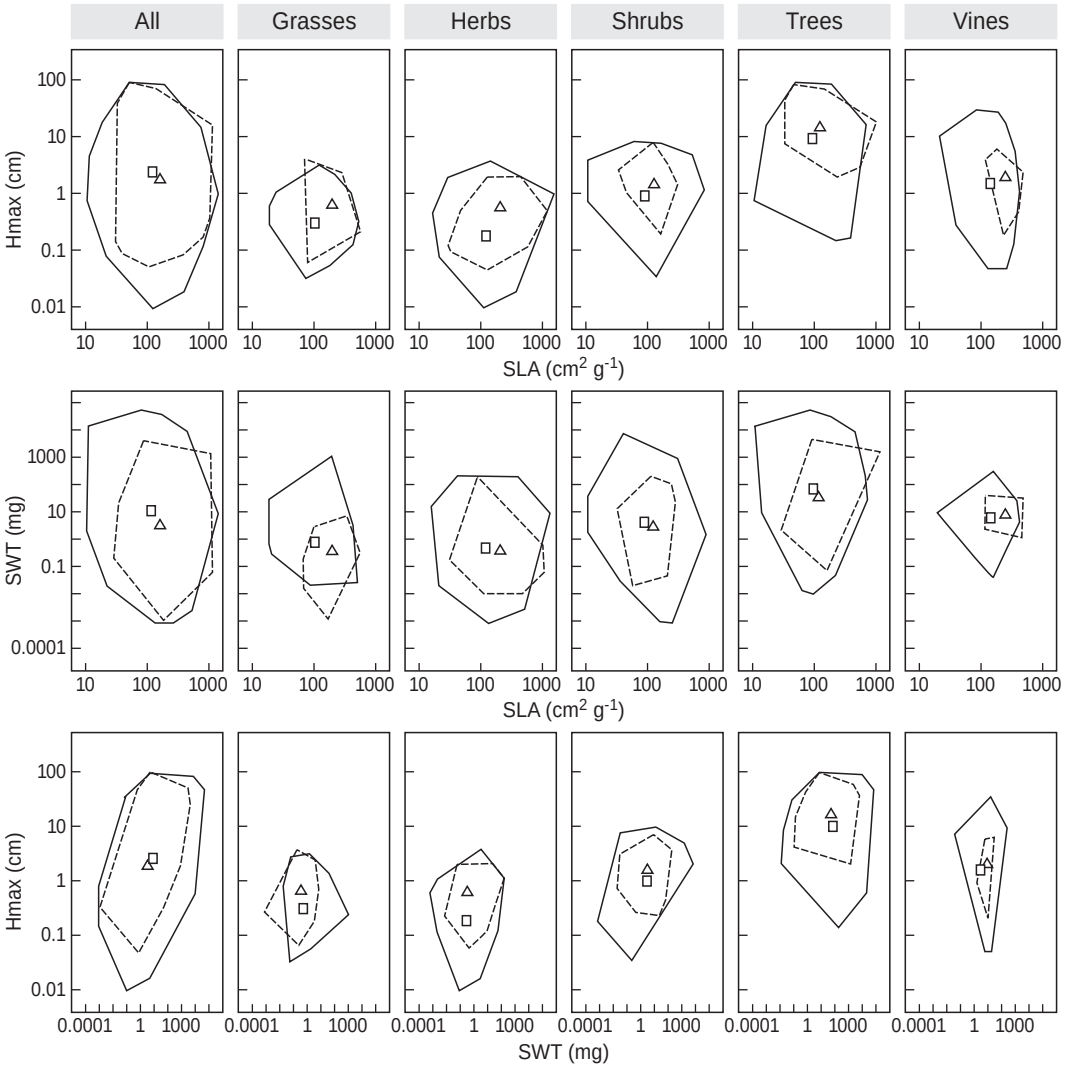

Figure 2. Minimum convex hull projections of native and alien species, showing the differences between these species-groups in the multidimensional trait space that they occupy, two traits at a time. Traits: specific leaf area (SLA, $cm^2\ g^{-1}$); maximum canopy height (Hmax, m) and individual seed size (SWT, mg). The polygons represent 2–dimensional crosssections of the convex hull volumes for each of the evaluated planes. Traits and axes were log10 transformed for analysis and representation. Measurements of functional richness represent the volume of each convex hull; trait divergence corresponds to the distance between centers of gravity (centroids); functional divergence corresponds to relative distance of alien and native species centroids from the community mean trait combination.

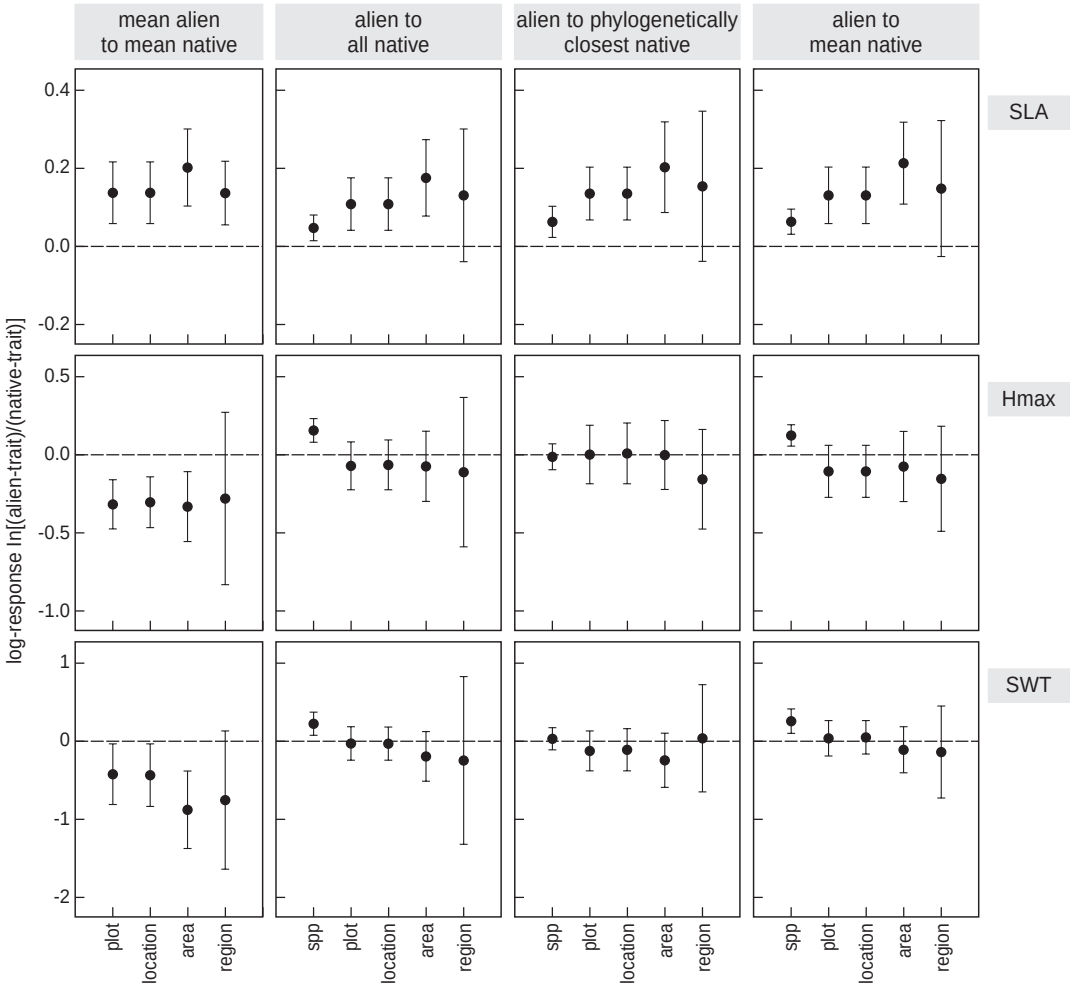

Figure 3. Log-response differences contrast a crossscales and comparision criteria for three species traits (Specific leaf area–SLA, typical maximum canopy height–Hmax, Specific seed Weight–SWT). Between group contrasts represent differences between i) average Alien to average native, ii) alien to all co-occurring natives, iii) alien to the phylogenetically closest native and iv) alien to the mean composition of all co-occurring natives. Points represent mean log-differentiation and whiskers represent the 95% confidence intervals

high species diversity are less likely to be invaded. This is often explained by the lower resource availability thought to occur in more diverse communities. The resource-enemy release hypothesis is also based on the idea that a plant community with unused resources (such as water, nutrients, space or light) will be more susceptible to introductions. In this case, fluctuations in the resource availability (due to stochastic variations in resource use or supply) will create windows of opportunity for invasions.

Test of this ideas using leaf traits important for plant carbon capturing strategies (SLA, Amass and Nmass), showed how performance of native and alien plants is associated to resource availability gradients. The evidence for this was the significant relation

between alien and native traits (and position along the carbon-strategy spectrum) and gradients of soil fertility and disturbance. However, our comparison of the difference in traits and carbon capture strategies of co-occurring alien and natives showed no relation to environmental and disturbance gradients (Fig. 4). Based on these results, we proposed that although higher resource availability benefits plant performance, these benefits are the same for both aliens and natives. This invalidates the hypothesis that especially high resource availability promotes invasions by allowing aliens to outperform natives due to differences in key traits that matter only under those conditions.

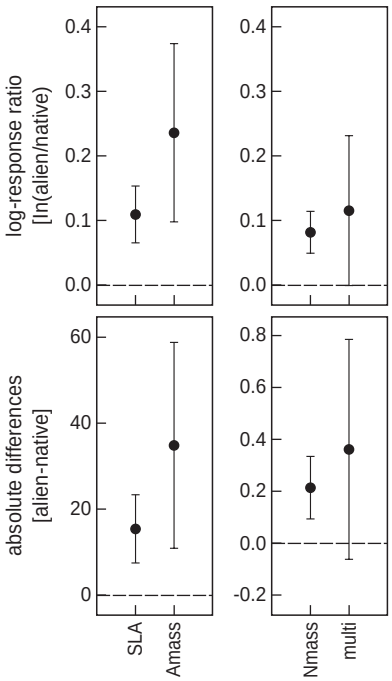

Figure 4. Differences between alien and native plants specific leaf area (SLA), foliar nitrogen per mass bases (Nmass), maximum photosynthetic rate per mass bases (Amass), and the position in the multi–trait leaf economics spectrum (Multi) between alien and native plants. Points mark the mean between group differentiation and whiskers above and below the point indicate the 95% confidence intervals.

It's possible that synergy between the biological setup and the environmental conditions is what determines whether or not an invasion will be successful. Although this couldn't be specifically tested in this project (due to lack of adequate information on the abundance or presence of enemies), it is possible that the escape form enemies by aliens, once introduce to a new area causes the differences between alien-native positions along the *"leaf economics spectrum"*. This will be especially the case for areas with high resource/disturbance rates. As experimental evidence provides a strong support for this idea, there is still a need for large scale field based con-specific comparisons, aiming to evaluate how alien species in high resource/ disturbance situations are more strongly released from enemies than those under lower resource/disturbance conditions.

The third dimension evaluated in this project is the relation between evolutionary process, and the mechanisms determining the success of colonizing species. Although most of research on the mechanisms underlying biological invasions has focused on the ecological explanations, several works have recently addressed the link between evolutionary patterns and alien success. These efforts have generally assumed two types of associations between evolutionary and niche divergence/ convergence patters: i) the degree of phylogenetic relatedness translates in to niche overlap, and ii) the niche of a species is fixed over ecological time scales. Together, these two associations are at the base of many population, community and macro ecological studies, and therefore has major implications for conservation biology/ecology.

The role of evolution on the success of colonization success of alien species has been formalized in several evolutionary hypotheses to explain invader success. In this work, two of the most important theories making this

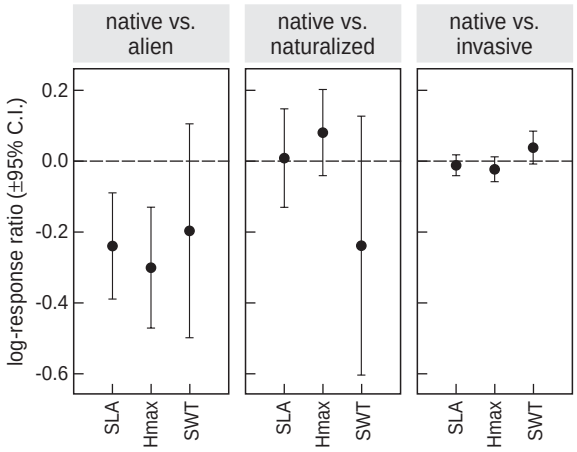

Figure 5. Weighted mean log-response ratios for conspecifics native alien/naturalized/invasive range comparisons. Evaluated traits are specific leaf area (SLA – $cm^2\ g^{-1}$), maximum canopy height (Hmax – m) and individual seed size (SWT – mg). The effect size is considered significant if the 95% condence interval does not overlap zero. From left to right, the numbers of records in each category were: 67, 103, 146, 51, 81, 121, 18, 27 and 30.

linking were examined: evolution of increased competitive ability and Darwin's naturalization hypothesis. In both of these hypotheses, the influence of evolutionary process on aliens' success might be in the form of competitive advantages when compared to co-occurring natives, or performance differences between the introduced and native range.

Our tests of the evolution of increased competitive ability hypothesis (i.e. changes between ranges) showed how traits of conspecifics are consistently similar between the native and introduced range (Fig. 5). These similarities were observed at both uni- and multidimensional trait spaces. This would suggest that traits are conserved along the trait spectrum, are little subject to evolutionary change. However, we do not imply that aliens do not have the potential to phenotypically or genetically adapt to the new conditions they might encounter in their new habitat in any trait. But we suggest that those traits that have been shown to be highly conserved over evolutionary time (e.g. wood density, seed mass, SLA, leaf life span) will be more conserved across ranges than those that are highly influenced by biotic, ecological or environmental gradients (e.g. canopy transpiration, rhizome re-sprouting, height, relative growth rate).

Based on the phylogenetic conservation of niches idea, Darwin's naturalization hypothesis was evaluated by comparing the relation between co-occurrence, phylogenetic relatedness and trait similarity of alien and native plants). Additionally the level of spatial variability on these relations was also assessed. Results form these two evaluations indicated that alien species show phylogenetic clustering (traits of co-occurring species are more similar than expected by chance) when compared to co-occurring natives. These trends are consistent for individual growth forms and different comparison criteria (i.e. all co-occurring natives, phylogenetically closest native and average native); and when comparisons controlled for spatial scale. This provides strong support for the importance of incorporating evolutionary patterns and the need to account for phylogenetic relationships when examining alien invasions, especially when the goal of the study is to predict which alien species might be successful.

Our work has aimed to link three separate lines of evidence (species invasiveness, community invasibility and evolutionary pat-

terns) that have received considerable attention independently. It is clear that if the ultimate goal is to predict invasion risks in the future (by a species or of an area) these three dimensions must be jointly accounted. The work presented here is a contribution to the long lasting quest for understanding the causes and mechanisms behind the species invasiveness-community invasibility continuum.

In conclusion, we suggest that determining successful introductions will require the evaluation of three community attributes: the level of functional similarity between natives and aliens; the degree of phylogenetic relatedness between these groups; and the resource availability-disturbance regime of the target community.

Effects of cattle and rabbit grazing on clonal expansion of spiny shrubs in wood-pastures

Christian Smit, Elisabeth S.Bakker, Emile M. Apol, Han Olff

Spiny shrubs protect non-defended plants against herbivores. Therefore, they play a role for the diversity in grazed ecosystems. While the importance of these keystone nurse-shrubs is presently recognized, little is known about the factors controlling them. This knowledge is required to understand the functioning of grazed ecosystems and for sustainable management.

We studied the effects of cattle and rabbits on clonal expansion of Blackthorn *Prunus spinosa* in two ancient wood-pastures in the Netherlands. At each site we set up five blocks in grassland perpendicular to the edges of mature *Prunus* thickets, each block containing three herbivore treatments: (1) open to cattle and rabbits, (2) open to rabbits, cattle excluded, (3) cattle and rabbits excluded (Fig. 6). We monitored the number and volume of Prunus ramets from 1998–2000 and again in 2003, three years after exclosure-removal to restore grazing.

For 1998–2000 ramet volume, but not ramet number, differed between treatments (Fig. 7). Ramet volume was highest when both cattle and rabbits were excluded. Ramet vol-

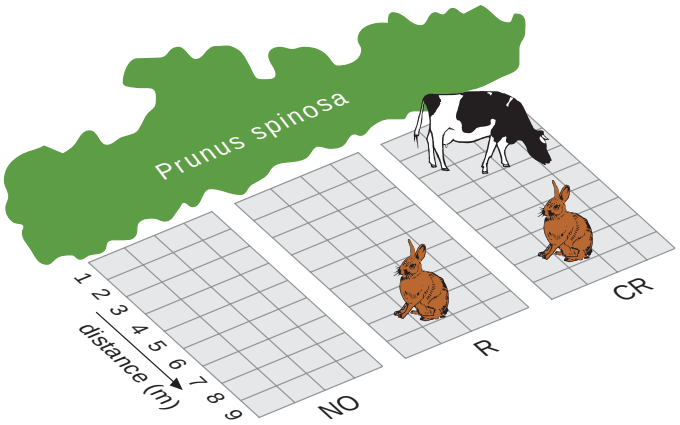

Figure 6. Schematic overview of experimental design of one block (15 x 10 m) in open grassland adjacent to a mature *Prunus spinosa* shrub, with plots subjected to cattle and rabbits (CR), rabbits (R) and no cattle and rabbits (NO).

ume did not differ between grazing by rabbits or cattle and rabbits combined, indicating that rabbits alone may be as effective in inhibiting clonal expansion as cattle and rabbits combined. Three years after exclosure-removal ramet number and volume had increased in all treatments. Number of ramets remained unaffected by (former) treatments. Ramet volume remained highest in the former cattle-plus-rabbits exclusion treatment, differing significantly from the 'open-to-cattle-and-rabbits' treatment. So, once successfully established during herbivore absence, further expansion is not prevented by cattle and rabbit grazing.

This study shows that vertebrate herbivory controls the keystone nurse-shrub in wood-pastures: combined cattle and rabbit grazing, and notably rabbits alone, inhibit expansion. Temporary herbivore absence allows expansion of ramets, which persists after herbivore-reappearance. Sustainable management of wood-pastures should allow spatial-temporal fluctuations of herbivore densities, leading to increased vegetation structure and associated biodiversity.

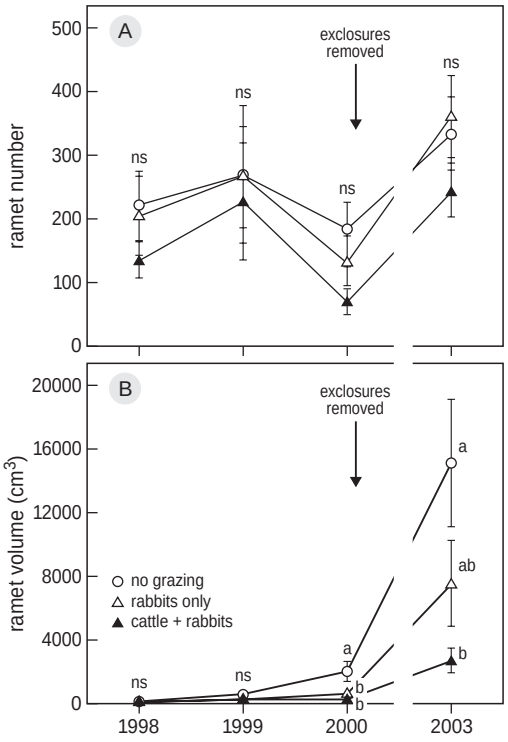

Figure 7. Number of ramets (A) and ramet volume (B) for the years 1998, 1999, 2000 and for 2003 (3 years after removal of exclosures). Depicted values are means ± 1se. Different letters indicate significant differences between treatments within years at $p < 0.05$.

Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought

Jofre Carnicer, Marta Coll, Miquel Ninyerola, Xavier Pons, Gerardo Sánchez and Josep Peñuelas

Climate change is increasing the frequency and severity of extreme climatic events, such as droughts, hurricanes, tornados, windstorms and floods. In Southern Europe and the Mediterranean basin, during the last decades climate change has lead to an abrupt increase in the frequency of severe drought events. A new study of the Community and Conservation Ecology Group (COCON) and the Global Ecology Unit of the Autonomous University of Barcelona (CREAF-CEAB-CSIC, UAB) has examined the consequences of increased drought impacts in European forests during the last 20 years. The study, conducted by the scientists Jofre Carnicer, Marta Coll, Miquel Ninyerola, Xavier Pons, Gerardo Sánchez and Josep Peñuelas, has been recently published in the journal

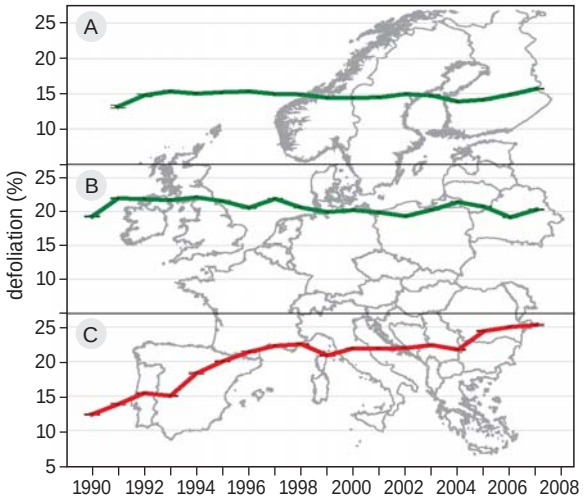

Figure 8. Increased defoliation of Southern European forests (red line, C) relative to the forests of Central and Northern Europe (green lines, A & B).

Proceedings of the National Academy of Sciences USA (PNAS). The results of the study are conclusive: due to increased drought impacts, Southern European forests have increased leaf losses and have doubled the defoliated area of tree crowns during the last two decades. Consequently, Southern European forests now show a widespread decline in tree crown condition in all forest tree species (see Figures 8 and 9). Moreover,

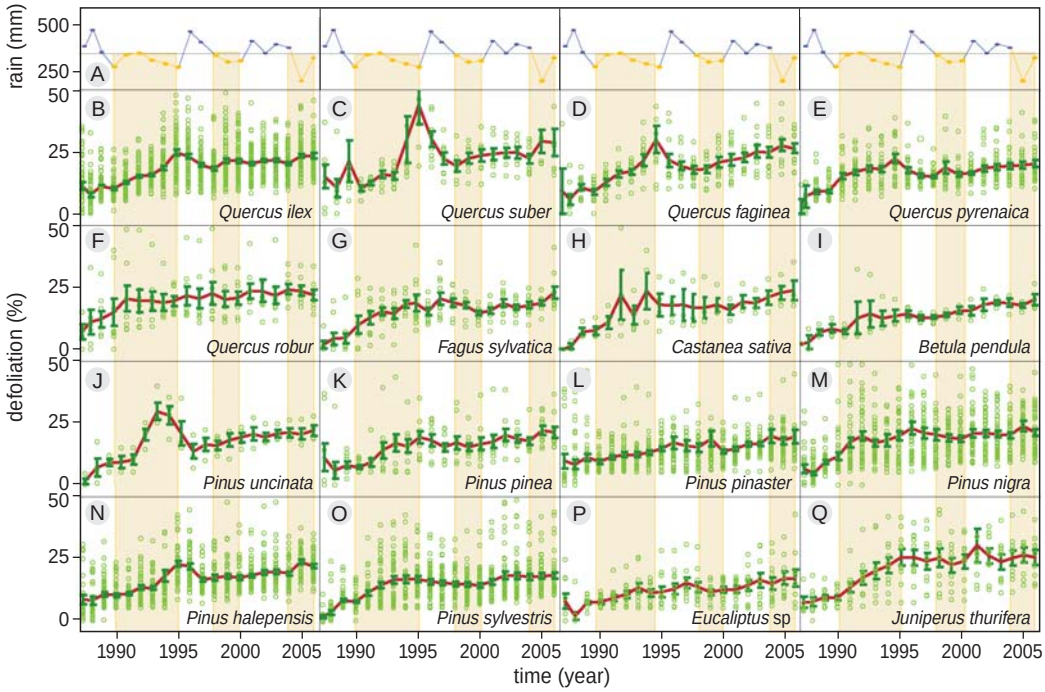

Figure 9. Increased defoliation in the main forest tree species of the Iberian peninsula.

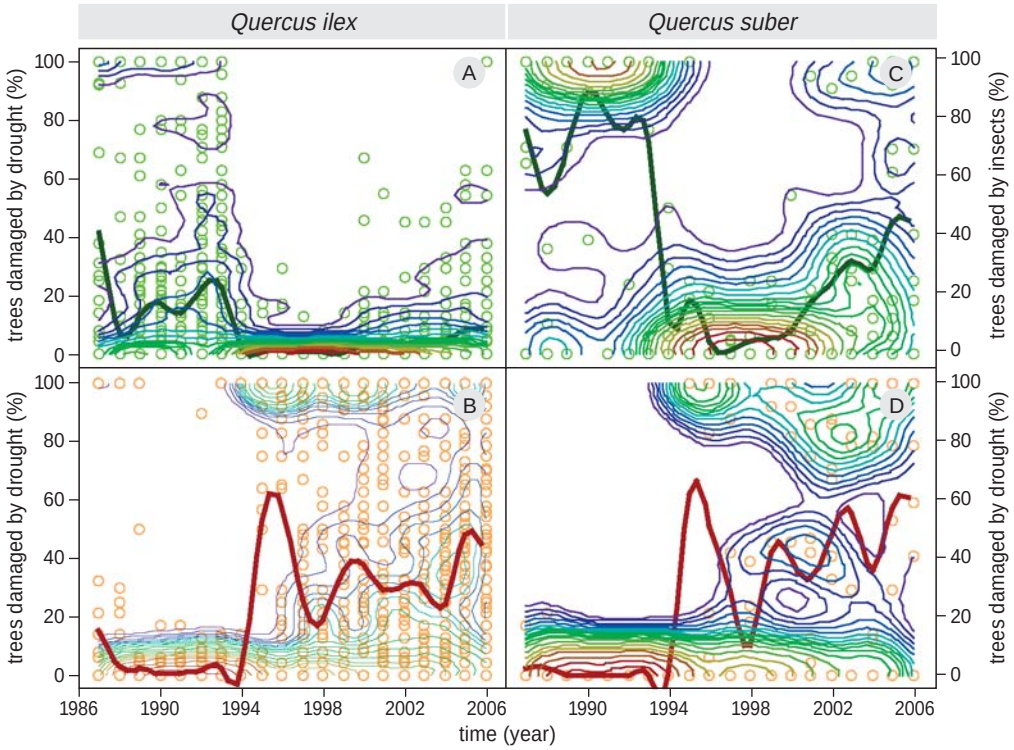

Figure 10. Disruption of insect and fungi communities (green lines; panels A & C) due to the effects of increased drought impacts (red lines, panels B & D) in Holm oak forests (*Quercus ilex*) and Cork oak forests (*Quercus suber*).

severe drought impacts have also disrupted the animal and fungal communities that rely upon trees for their alimentation, causing reduced fungal and insect abundances during several years after drought (Figure 10) and potentially increasing the extinction risk of the many rare and endangered species inhabiting this highly diverse area. Notably, the Mediterranean basin currently stands as a hot spot of animal and vegetal biodiversity and increased drought impacts are one of the major threats in this area.

The study highlights that the reported forest declining trends may easily feedback to the climate system, because forests play an important role in carbon sequestration (proximately one third of the atmospheric CO_2 emissions are annually captured by forests). Moreover, forests play a key role cooling the environment during hot summer periods through delayed leaf water transpiration of previous spring and winter rainfalls. The reported increased defoliation levels suggest a reduced cooling capacity of Southern European forests during extreme hot summers. Therefore, this diminished transpiration capacity of increasingly defoliated forests may in turn facilitate the emergence of more intense and long-lasting of extreme summer heat waves in the near future.

Peak oil and climate change: the urgent need for a transition to a non-carbon emitting society

Jofre Carnicer, Josep Peñuelas

The strong environmental effects of greenhouse gas emissions derived from oil use and the negative socio-economic consequences of future oil scarcity make it urgent to shift to alternative affordable energy sources. A recent assessment of the International Energy Agency, an OECD prestigious institution, alerts that oil shortage and increased energy costs can easily be an immediate reality after the current financial crisis if massive and strategic investments in oil industry are not rapidly and massively implemented.

Multiple economic, scientific, technological and political pathways should be implemented to achieve this global energy transition. States should empower their national strategies to improve the efficiency in energy generation, transmission and consumption and thus reduce progressively carbon emissions. States should also facilitate the massive deployment of renewable energies and public transport, promote the progressive electrification of the car industry, and globally shift to sustainable strategies in many other economic sectors. At the international level, governments should rapidly promote multilateral and bilateral cooperative agreements on energy and climate policies. In addition, states might promote the creation of a United Nations international programme to facilitate and coordinate a world-wide ordered and non-traumatic transition to low-carbon and energy-efficient economie. This UN international programme could develop or facilitate multilateral regulatory agreements to avoid the emergence of speculative dynamics and volatility on oil prices that ultimately damage economic stability and increase ongoing global food-security crisis. Finally, I advocate for a much greater scientific effort urgently placed on the interactions between peak oil, climate change and global society change. The scale, urgency and severity of peak oil and climate change mean that no action is too small to matter, too large to contemplate, or too soon to begin. There is not much time left.

Faster returns on 'leaf economics' and different biogeochemical niche in invasive compared with native plant species

Josep Penuelas, Jordi Sardans, Joan Llusia, Susan M. Owen, Jofre Carnicer, Thomas W. Giambelluca, Enrico L.Rezende, Mashuri Waite, Ulo Niinemets

Plant-invasive success is one of the most important current global changes in the biosphere. To understand which factors explain such success, we compared the foliar traits of 41 native and 47 alien-invasive plant species in Oahu Island (Hawaii), a location with a highly endemic flora that has evolved in isolation and is currently vulnerable to invasions by exotic plant species. Foliar traits, which in most cases presented significant phylogenetic signal, i.e. closely related species tended to resemble each other due to shared ancestry, separated invasive from native species. Invasive species had lower leaf mass per area and enhanced capacities in terms of productivity (photosynthetic capacity) and nutrient

capture both of macro- (N, P, K) and microelements (Fe, Ni, Cu and Zn). All these differences remain highly significant after removing the effects of phylogenetic history. Alien-invasive species did not show higher efficiency at using limiting nutrient resources, but they got faster leaf economics returns and occupied a different biogeochemical niche, which helps to explain the success of invasive plants and suggests that potential increases in soil nutrient availability might favor further invasive plant success.

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Hopcraft, J.G.C. (2010): Ecological implications of Food and Predation Risk for Herbivores in the Serengeti. PhD thesis, University of Groningen, the Netherlands

Ecophysiology of Plants

Group leader Prof. dr. J.T.M. Elzenga

Composition of the group in 2010:

Tenured Staff	**source**	**fte**	**period**
Prof. dr. J.T.M. Elzenga	RUG	1.0	
Dr. L.J. de Kok	RUG	1.0	
Post-docs			
Dr. J. Stefels	NWO-ALW	0.8	100101-121231
Dr. N. Sujeeth	EU-FP7	1.0	100401-130401
Dr. J.H. Venema	EL&I	0.8	100401-140401
PhD Students			
T. Aghajanzadeh	Razi Med. Diag. Lab.	1.0	101101-141031
C. Fritz	St Peat Research	1.0	061201-111201
I.A. Neven	NWO-ALW	1.0	060215-110115
W. Patberg	NWO-ALW	0.8	060601-101001
C. Schmitz	Robert Bosch Stiftung	1.0	041001-081001
M. Shahbaz	NUFFIC bursary	1.0	070901-110901
J. Spoelder	HLB Laboratory	0.6	101201-151201
F. Zaki Mostafa Ali	Egyptian Government	1.0	070201-110201
Technical/Administrative Staff			
secretary			
J. Nuijten-Tjalkes	RUG	0.4	
administrator			
G.P. Telkamp	RUG	1.0	
research analist			
F.S. Posthumus	RUG	1.0	
M. Staal	RUG	1.0	
C.E.E. Stuiver	RUG	0.7	
Guest researchers			
Dr. I. Stulen	Groningen, NL		
Martin Reich	Univ.of Frankfurt		

Introduction

Movement of water, nutrients and organic compounds in and out of plants or algae, between plant organs and between the cell wall compartment and the cytoplasm of a cell, are processes that define the functioning of an organism. The affinity of the uptake system for a limiting nutrient from the soil determines the outcome of the competition between species; the signalling molecules released by the roots shape the composition of the microbial community around the roots and facilitate the establishment of the symbiosis with beneficial bacteria and with mycorrhizal fungi; pathogenic bacteria trigger ion movements across the cell membrane, involved in the anti-bacterial response of the plant determining the susceptibility of a plant to the pathogen, the release of organic sulfur compounds by algae affect temperature on a global scale. With modern biophysical techniques the fluxes of organic compounds and ions can be quantified. The Ecophysiology of Plants group develops new techniques that enable us to measure with higher precision the fluxes of nutrient ions and climate active compounds, on physiological and ecological relevant scales. Although the measurements are sometimes carried out on a scale as small as a single vacuole or a root tip, or involve samples collected during an expedition to Antarctica, the main goal always remains to understand the functioning of the whole organism in its, often variable, environment.
Plants are capable to respond to changes in their environment by changing growth pattern, adjust their physiology, resorb and form roots, accumulate defensive compounds or osmotically active, compatible solutes. This adaptive behaviour of algae and plants in turn can affect their environment, sometimes on a global scale.

Our approach, using advanced biophysical techniques to measure processes that are essential to plant functioning in a changing environment, and interpreting these in a whole plant context, is applied in two areas of interest:

1. Determining the interaction between global change factors and plant/algal performance: DMS release by algae, the effect of CO_2 levels on Sphagnum growth and algal photosynthesis, and the effect of atmospheric sulfur compounds on plant performance. In these projects effects of climate change and changes in the atmospheric composition on ecologically important processes are studied. Changes in plant growth due to increased CO_2 levels and the effects of atmospheric sulfur and nitrogen compounds on plant nutrient demand are themes that have a long history in the Plant Ecophysiology group. DMS is one of the so-called negative greenhouse gasses: it can function as a condensation nucleus, inducing cloud formation that has a cooling effect on a global scale. The production, release and subsequent hydrolysis of DMSP by algae, is the main source of DMS. The conditions that stimulate or inhibit the production of DMSP are, however, still not fully understood. Since Sphagnum bogs are the only terrestrial ecosystem that can function as a carbon-sink over geological periods, the establishment and/or restoration of these bogs can ameliorate the CO_2 production due to burning of fossil fuel.

2. Describing, understanding and possibly optimizing the acclimative characteristics of plants to suit the changing requirements of growing crop plants. Examples of such projects are: plant performance under suboptimal temperature, mechanisms of salt-tolerance in maize, molecular

basis of root architecture and uptake characteristics, and effects of plant-microbe interaction on plant physiology and development. These projects are characterized by a strong interest of commercial partners. By focussing the physiological research on commercial crop plants the combination of challenging scientific questions and practice-oriented solutions has proved to be inspiring and productive.

Overview of academic results

Physiology and ecology of Sphagnum bogs

Wouter Patberg, Gert Jan Baaijens[1], Fons Smolders[2], Ab Grootjans[2,3] and Theo Elzenga

(1. BaaijensAdvies, Dwingeloo; 2. Department of Aquatic Ecology and Environmental Biology, Radboud University Nijmegen; 3. Center for Energy and Environmental Studies IVEM, University of Groningen)

Bogs, which are dominated by the bryophyte Sphagnum, are the only terrestrial ecosystems that continuously can store CO_2. In the Netherlands, large areas in the eastern provinces were once covered with extensive peatbogs. The bogs have to a large extent disappeared by cutting the peat for heating and by draining the bogs to allow agriculture. To restore Sphagnum bogs the area is re-wetted by cutting trees to reduce evapotranspiration and by filling drainage ditches. These measures to increase the groundwater level have not always the desired result: sometimes Sphagnum growth is restored and dominates the vegetation, but the growth of Sphagnum is limited and grasses have the overhand. And sometimes the raised water table leads to small lakes with open water. In the Dwingelderveld, a large nature reserve in the Netherlands the return of Sphagnum dominated bogs is related to carbonate-rich water flowing into the hollows where the bogs are forming. This carbonate-rich groundwater results in a high CO_2 concentration in the upper layer of het Sphagnum bog. This is the layer where the physiologically most active part of the Sphagnum plants is found. Most photosynthetic activity and growth of the plants takes place in the top layer. The high CO_2 concentration in this layer is necessary as photosynthetic demand for CO_2 of Sphagnum is being met by mere diffusive supply from the atmosphere. The reason for this is the thin water layer that surrounds the Sphagnum leaves presents a barrier to diffusion. In the original, mature Sphagnum bog, where the thin, living layer of Sphagnum mosses grows on top of a thick layer of organic material, the atmospheric CO_2 is being supplemented by CO_2 from the slow, but steady, decay of the thick peat deposit layer. Since this peat layer has been removed, Sphagnum only seems to be able to return in locations where the CO_2 supply from a different source (in this case the carbonate-rich groundwater) is kept at a high level.

In the Dwingelderveld, carbonate-rich groundwater is found in hollows that are located in the erosion gullies that were formed in the aftermath of the ice-ages and that are characterized by a water impermeable boulder clay layer (Figure 1).

This layer facilitates water flowing from the surrounding area into the bog. This water will be enriched in carbonate by the prolonged contact with the mineral soil. The acids produced by the Sphagnum vegetation

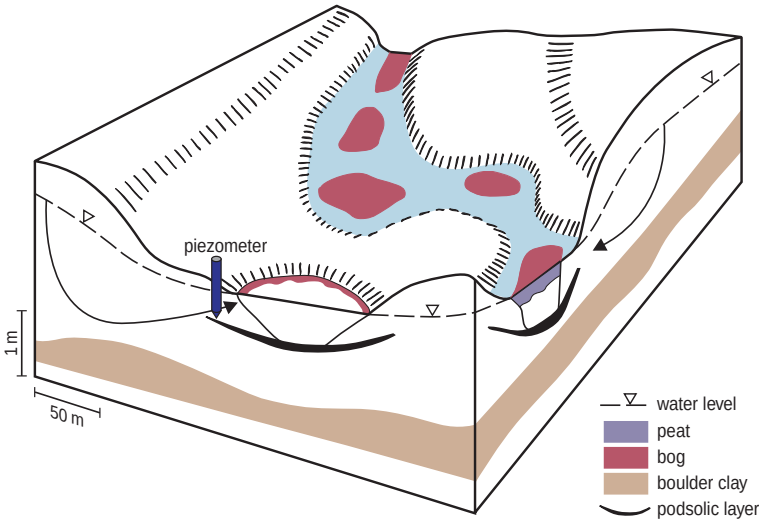

Figure 1. Diagram of the gully system in the Dwingelderveld National Park. The small lakes located in the gullies do receive more carbon-rich groundwater than the lakes that are outside the gully system. The development of a Sphagnum-dominated bog is more likely to be successful in the hollows that are receiving additional CO_2 from the carbon-rich groundwater.

will, when they react with the carbonate/bicarbonate in the groundwater, result in the formation of the high CO_2 levels needed for growth the mosses. Ultimately, the physiological, photosynthetic characteristics of the Sphagnum mosses and the landscape features determine the success (or failure) of restoration measures.

Sphagnum bogs are also characterized by a very low availability of nutrients. Although the hydrological characteristics of the deeper water layers prove to be very important for the establishment and growth of a bog, most of the nutrients input in a bog is atmospheric. In the more heavily industrialized regions of the world, like the Netherlands, the atmospheric deposition of nitrogen is high enough to sustain growth. However, in more pristine areas the atmospheric deposition is not enough to support the observed growth and accumulation of organic material. Once nutrients are brought into a bog by precipitation and incorporated in the system, it is therefore essential that they are efficiently recycled. In Sphagnum bogs nutrients are being released by decomposition of organic material (peat) in the deeper layers, while growth of the Sphagnum plants occurs in the so-called capitulum, the leafy structure at the top of the plant. Nutrient uptake by Sphagnum from these deeper layers and internal transport to the capitulum is a very slow process. An alternative mechanism is the mixing of deeper, nutrient rich water and nutrient poor, superficial water, by a process that has been described about 10 years ago, as a theoretical possibility: vertical mixing by convection cells that arise when the day and night temperatures differ sufficiently. Convection cells can bring nutrients to the surface and thus make them available to the growing capitula. Work done the last two years has demonstrated the occurrence in a natural situation and, moreover, proofed that nutrients are transported to the surface layers and uptake of nutrients by Sphagnum plants is facilitated by convection cells generated by day-night temperature differences. An analysis of day and night temperatures throughout the year at locations where Sphagnum bog are found shows that convection cells might be the prime transport mechanism for recycled nutrients supporting Sphagnum growth world wide.

Inorganic carbon uptake by Southern Ocean phytoplankton in a changing world

Ika Neven, Jacqueline Stefels, Theo Elzenga, Hein de Baar

The fixation of dissolved inorganic carbon (DIC) by photosynthetic microalgae is know to play an important role in the sequestration of anthropogenic carbon by means of a process called the biological pump. Dying and dead phytoplanktonic cells sink out of the euphotic zone and export the carbon they have photosynthetically fixed to the deep ocean, where it is stored for centuries before being released to the atmosphere again. Consequently, it is important for the predictions of future atmospheric CO_2 to determine whether marine primary productivity itself is susceptible to changing atmospheric CO_2 levels. In the last decade it has been shown, that Antarctic primary productivity can be severely reduced by a co-limitation of dissolved iron (dFe) and light. However, due to the special chemistry of DIC in seawater, the concentration of CO_2 – which is the carbon species for photosynthetic fixation in the Calvin cycle – might pose another limiting factor. An increase of DIC in seawater due to anthropogenic activity, and hence an increased concentration of CO_2 might have a significant effect on future phytoplanktonic production.

In this project the effects of rising anthropogenic CO_2 levels on the ecophysiology of Southern Ocean phytoplankton are evaluated by means of CO_2 manipulation experiments with single species under controlled laboratory conditions and field experiments with natural phytoplankton communities from the Southern Ocean. Six shipboard CO_2 manipulation experiments were performed in the Atlantic sector of the Southern Ocean from February until April 2008. Phytoplanktonic growth, community composition and DIC acquisition was examined during 10 – 14 d in response to the following CO_2 scenerios: last glacial maximum (~190 µatm), present (~380 µatm) and future high CO_2 (~750 µatm). No effect on the community composition was observed (data not shown). However, phytoplankton accumulated significantly more particulate organic carbon (POC) when grown under high CO_2 concentrations and nutrient replete conditions in comparison to phytoplankton grown at present or low CO_2 concentrations (Figure 2A).

At 750 µatm CO_2 phytoplankton accumulated on average 38% more POC than under

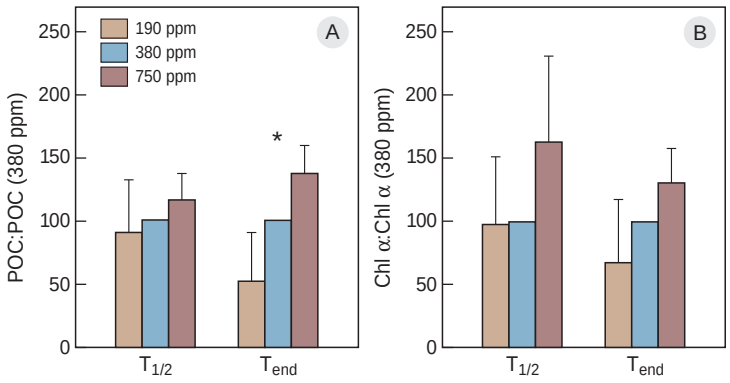

Figure 2. Biomass development in all six experiments expressed as percentage of the control experiment at 380 ppm CO_2.
A. Particulate organic carbon; B. Chlorophyll-*a*. The error bars represent standard deviations.

present CO_2 conditions. At 190 µatm CO_2 phytoplankton stored on average 47% less POC than at 380 µatm CO_2. The same trend although not significant was observed when comparing the measured chlorophyll-a concentration between the different treatments (Figure 2B).

However, the differences in the accumulation of POC could not be explained by the preference of carbon species, as phytoplankton did not increase the uptake of CO_2 in response to higher CO_2 concentrations in seawater. Instead, phytoplankton upregulated HCO_3^- uptake when incubated in all treatments. This might be the result of nutrient- and light-replete conditions in the incubations enabling phytoplankton to invest in DIC uptake without any energy limitations. Our experiments further suggest that Southern Ocean microalgae could accumulate more carbon under future high CO_2 conditions and nutrient replete conditions. Whether this has the potential to alter the biological tissue pump in the Southern Ocean needs to be examined.

Development of methods for the analysis of sulfur compounds in sea ice

Jacqueline Stefels[1], Gauthier Carnat[2], John W.H. Dacey[3], Thomas Goossens[2], J. Theo M. Elzenga[1], Jean-Louis Tison[2]

(1. University of Groningen, Netherlands; 2. Université Libre de Bruxelles, Belgium; 3. Woodshole Oceanographic Institution, USA)

High concentrations of the climate-active, biogenic gas dimethylsulfide (DMS) in surface waters affected by melting sea ice have been regularly reported. Whether these were the result of direct release from ice or of a boost of the in-situ production by phytoplankton was never quantified. This was largely due to the fact that the analysis of reduced sulfur compounds in sea ice is not easily accomplished. As a consequence, making budgets of the sulfur cycle between the ice-ocean-atmosphere interfaces has not been possible.

The hypothesis that DMS can be produced in sea ice was triggered by early observations of extremely high concentrations of dimethylsulfoniopropionate (DMSP) in sea ice. DMSP is the precursor of DMS and is produced by microalgae. The concentration of algae in sea ice can be extremely high and variable (Figure 3). These ice algae have physiologically adapted to the conditions of high salt concentration and low temperature that often prevail within ice. Such conditions are known to be favourable for increased production of DMSP by algae, as this compound is a compatible solute that has a role in cryoprotection and osmotic acclimation at high intracellular concentrations.

For most biochemical analyses melting of ice cores is needed. Depending on temperature, the salinity of the brine inclusions that contain the algal community can be more than 3 times seawater salinity, whereas bulk salinity after melting is close to freshwater values. Therefore, without taking precautions, melting of ice samples results in a salinity shock that is detrimental for the organisms living inside brine channels. It will affect ice-algal physiology and even may result in cell lysis. As a consequence, the compounds of

Figure 3. Algal biomass in sea ice is extremely heterogeneous. Discrete layers of chlorophyll result in chlorophyll concentrations that vary over a range of three orders of magnitude. Bottom layers mainly consist of diatoms.

interest are released from the cells and are potentially converted by exoenzymes. This has been, and still is, a problem associated with sea-ice research, and needs to be checked with every new technique. Reduced sulfur compounds are particularly vulnerable, since these compounds are subject to conversion in many pathways and processes within the microbial food web that may change the original configuration of compounds when melting artifacts occur.

We have developed two new methods to analyze DMS and DMSP in sea-ice cores accurately. The first, describes the dry-crushing method, which has its focus on the volatile compound DMS. A sub-sample of deeply frozen ice is crushed in a stainless steel vessel and the released gas phase is analyzed directly for DMS. The remaining ice is subsequently analyzed for its total DMSP content. The second method involves a melting procedure during which the conversion of compounds is monitored by adding differently deuterated isotopes of DMS and DMSP. Natural concentrations and stable isotopes of DMS and DMSP are simultaneously analyzed on a Proton-Transfer-Reaction Mass Spectrometer (PTR-MS). Loss and conversion rates of the artificial isotopes are used to reconstruct the original concentrations of DMS and DMSP in ice and give important information on potential dynamical processes in sea-ice communities.

With the dry-crushing method we were able to resolve accurate DMS and DMSP profiles even in ice cores stored deeply frozen for two years. When direct processing and analysis of the samples is possible, the isotope-addition method has the advantage that besides providing concentrations of S-compounds (Figure 4A and B), it is suitable to determine process rates within the S-cycle (Figure 4C). In the example shown here, some interesting observations can be made that illustrate the power of this method. The coincidence of elevated hexa:fuco ratios, as marker for Haptophyte algae (Figure 4D), and high DMSP-conversion activity (Figure 4C), suggests that this DMSP-lyase activity is associated with *Phaeocystis antarctica*, which is a well-known producer of DMSP and also has the ability to enzymatically convert DMSP into DMS. This conclusion is supported by the elevated DMSP-to-chlorophyll-a ratios observed in the upper half of the ice core: on average 240 mmol/g, whereas ratios in the bottom 30cm decreased from 90 to 25 mmol/g. The

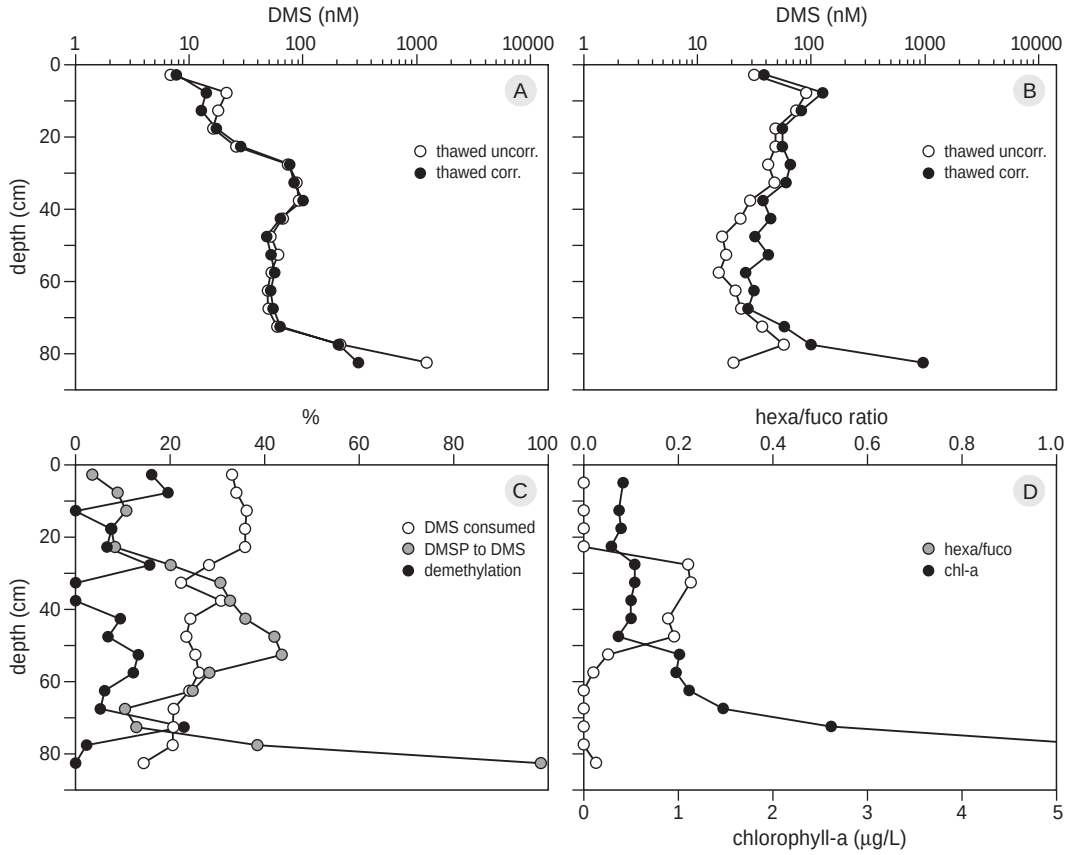

Figure 4. Ice-core profiles of S-compounds, conversion processes and pigments in an ice core from the Weddell Sea (Antarctica), taken during the ISPOL expedition, Dec 2004. Uncorrected and corrected concentrations using isotope conversions of A. DMS and B. DMSP. C. Percentage of added isotope, converted or lost during the melting period. D. Concentrations in ice of chlorophyll-*a* and ratios of 19'-hexanoyloxyfucoxanthin to fucoxanthin as marker of Haptophyte algae. Chlorophyll-*a* concentrations in bottom sections increased to 48 μg L^{-1}.

high ratios in the upper half are also indicative for the presence of Haptophyte algae.

These results show that in order to investigate ecosystem processes and dynamics within the sulfur cycle, stable isotope additions are a very powerful tool. In combination with other biological and chemical parameters provides, this method provides the means for interpreting dynamic processes within the sulfur cycle and evaluate the results in the context of physiological or community adaptation to the physicochemical environment.

Whole plant regulation of the uptake and distribution of sulfate in Chinese cabbage (*Brassica pekinensis*)

L.J. De Kok, C. E.E. Stuiver, F.S. Posthumus, M. Shahbaz & M.J. Hawkesford*

*(Plant Science Department, Rothamsted Research, Harpenden, U.K.)

Sulfur is an essential macronutrient for plants, which content varies between plant species and ranges from 0.1 to 2 % of the dry weight. Sulfate taken up by the roots is the primary sulfur source for plants, but it needs to be reduced prior to its metabolism into organic sulfur compounds (Figure 5). In polluted areas dry and wet deposition of atmospheric sulfur gases (*viz*. SO_2, H_2S) may also substantially contribute to plant sulfur nutrition, and may affect the uptake of sulfate by the roots.

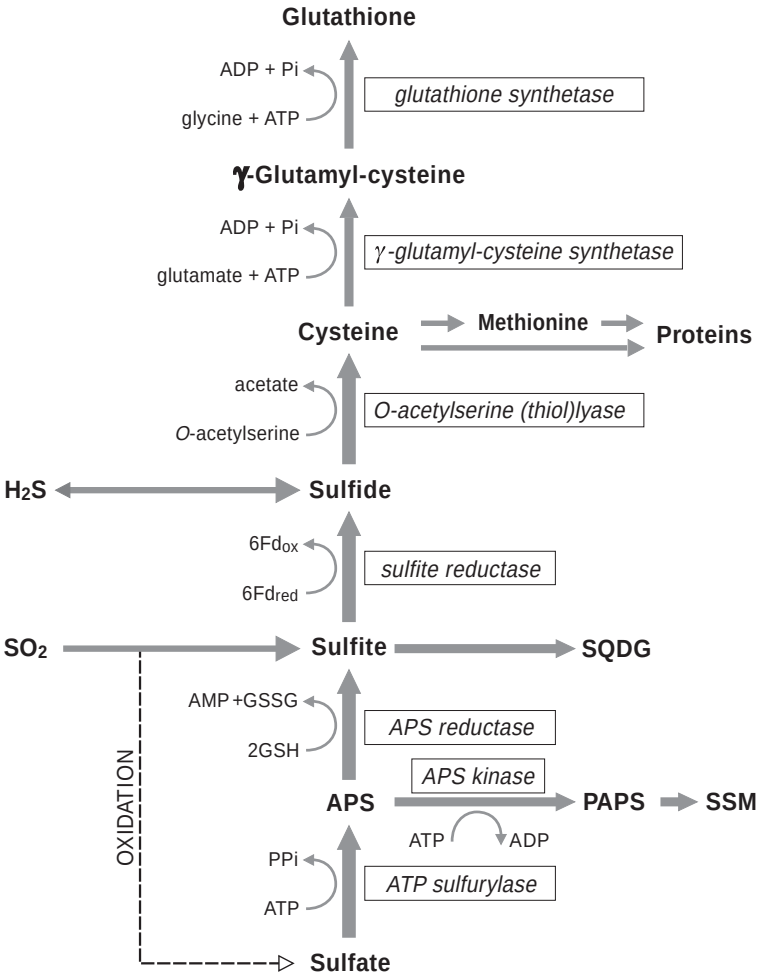

Figure 5. Sulfur metabolism in plants. APS, adenosine 5'-phosphosulfate; Fdred, Fdox, reduced and oxidized ferredoxin; GSH, GSSH, reduced and oxidized glutathione; PAPS, adenosine 3'-phosphate 5'-phosphosulfate; SQDG, sulfoquinovosyl diacylglycerol; SSM, secondary sulfur metabolites.

Brassica species are characterized by their high growth rate and sulfur demand and their seedlings may require an overall sulfate uptake of up to 40 μmol g^{-1} fresh weight root day^{-1} in order to maintain their growth. The uptake, transport and distribution of sulfate in the plant are mediated by different sulfate transporter transmembrane proteins. *Brassica* species contain 12–14 different sulfate transporter genes and the sulfate transporter family has been classified in up to 5 different groups according to their cellular and subcellular expression and possible functioning. In Brassica, the Group 1 sulfate transporters Sultr1,2 appeared to be responsible for the primary uptake of sulfate by the root, whereas Sultr1,1 was only expressed upon sulfate deprivation.

Similar to previous observations with curly kale (*Brassica oleracea*), the uptake of sulfate by Chinese cabbage was controlled by the sulfur demand for growth and modulated by the external sulfate concentration. If Chinese cabbage was grown at various sulfate concentrations ranging from 5 to 100 μM, plants were able to maintain their biomass production and growth rate (on average 0.34 g g^{-1} FW plant day^{-1}) even at 5 or 10 μM (Table 1). The latter sulfate concentrations are close to the Km value of the Group 1 high affinity sulfate transporters. The sulfate uptake capacity of the root was increased at ≤ 25 μM up to 2.5-fold at 5 μM sulfate (Table 1). The mRNA abundance of Sultr1;2, the constitutively expressed high affinity sulfate transporter in the root of Chinese cabbage, was hardly affected by the sulfate concentration in the root environment (Figure 6). Sultr1;1 was hardly expressed in roots of Chinese cabbage, but its mRNA abundance was only slightly enhanced at ≤ 25 μM sulfate (Figure 6). Moreover, the contents of sulfate and thiols in

Table 1. Impact of external sulfate concentration (5, 10, 100 μM) on biomass production, sulfate and thiol content, and sulfate uptake capacity in Chinese cabbage. Seedlings were grown on a 25% Hoagland nutrient solution at various sulfate concentrations for 4 days. Data on biomass production (g FW) and shoot to root ratio (S/R) represent the mean of 2 experiments with 12 measurements with 3 plants in each (± SD). On average plant growth rate was 0.34 g g^{-1} FW day^{-1}. Sulfate content (μmol g^{-1} FW) represents the mean of 2 experiments with 2 to 3 measurements with 6 plants in each (± SD). Thiol content (μmol g^{-1} FW) represents the mean of 3 measurements with 6 plants in each (± SD). Sulfate uptake capacity (μmol g^{-1} FW root h^{-1}) was measured at 25 and 500 μM sulfate and represents the mean of 3 measurements with 3 plants in each (± SD). Different letters indicate significant differences between treatments ($p \leq 0.01$, Student's t-test).

Sulfate [μM]	5	10	25	100
Biomass production				
Shoot	0.150 ± 0.018a	0.154 ± 0.022a	0.159 ± 0.023a	0.161 ± 0.017a
Root	0.030 ± 0.005a	0.029 ± 0.004a	0.027 ± 0.004a	0.029 ± 0.004a
S/R	5.0 ± 0.6a	5.5 ± 0.7a	5.9 ± 0.6ab	5.6 ± 0.6a
Sulfate content				
Shoot	13.8 ± 2.9a	13.3 ± 3.3a	14.3 ± 3.3a	13.0 ± 3.1a
Root	6.4 ± 0.5a	6.8 ± 0.4a	8.2 ± 0.6b	8.2 ± 0.2b
Thiol content				
Shoot	0.49 ± 0.03a	0.50 ± 0.04a	0.50 ± 0.03a	0.49 ± 0.03a
Root	0.37 ± 0.02a	0.43 ± 0.04a	0.43 ± 0.02ab	0.39 ± 0.09a
Sulfate uptake capacity	3.96 ± 0.41c	3.34 ± 0.33c	2.14 ± 0.30b	1.59 ± 0.13a

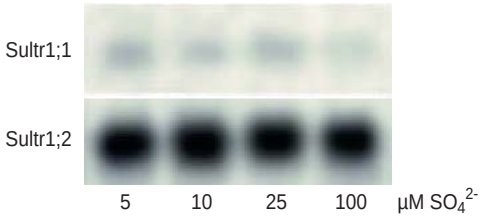

Figure 6. Impact of external sulfate concentration (5, 10, 25 and 100 mM) on mRNA abundance of the sulfate transporters in Chinese cabbage roots (see for experimental details Table 1).

both root and shoot of Chinese cabbage were hardly affected at low sulfate concentrations in the root environment; there was only a slight decrease in the sulfate content of the root at ≤ 10 μM (Table 1). From studies on prolonged sulfate deprivation it is evident that in addition to an increased uptake, the overall plant uptake capacity might also be enhanced by the change in biomass partitioning in favor of that of the root. However, in Chinese cabbage the shoot to root ratio remained unaffected at 5 and 10 μM sulfate, even upon more prolonged exposure (Table 1).

The sulfate transporters may be regulated at a transcriptional, translational and/or post-translational level, which might be signalled or mediated by sulfate itself and/or by products of the assimilatory sulfate reduction pathway (e.g. sulfide, cysteine and/or glutathione. Similar to other observations with *Brassica*, there was no clear relation between the overall tissue levels of sulfate and thiols and the activity of the sulfate transporters in Chinese cabbage upon variation in sulfate supply to the root. There was in Chinese cabbage no direct relation between the mRNA abundance of Sultr1;2, the primary sulfate transporter in the root and the sulfate uptake capacity.

In leaves of Chinese cabbage, Sultr4;1 was the sole constitutively expressed sulfate transporter present and its expression was enhanced upon direct incubation of the tissue at ≤ 8 mM and decreased at higher sulfate concentrations. Sulfate-deprived leaves were characterized by a high expression of Sultr1;2, Sultr4;1, Sultr4;2 and APS reductase. The high expression was down-regulated upon incubation of the leaf tissue at >2 mM sulfate, whereas at 20–30 mM their expression was quite similar to that of sulfur-sufficient leaf tissue. In both sulfur-sufficient and sulfur-deprived leaf tissue there was an accumulation of sulfate upon incubation nearly linear with the sulfate concentration. The thiol content of leaf discs was slightly enhanced upon incubation at high sulfate concentrations. Evidently, in leaf tissue of Chinese cabbage the overall *in situ* leaf tissue sulfate rather than the thiol concentration was of great significance in the regulation of expression of the sulfate transporters and APS reductase, the key regulating enzyme in the sulfate reduction pathway (Figure 5). The expression of Sultr1;2, Sultr4;1, and APS reductase might be controlled by the *in situ* cytosolic sulfate concentration in direct interaction with the sulfate reduction in the chloroplasts. The affinity of ATP sulfurylase for sulfate appears to be rather low (K_m for sulfate ranges from 0.5-3 mM) and the *in situ* sulfate concentration in the chloroplast at the ATP sulfurylase site may play a significant role in the rate of sulfate reduction.

On basis of the current knowledge and commonly applied research approaches, it is still unclear as to what extent measuring changes in concentrations of potential signal compounds and activity of the sulfate transporters, provides sufficient insight into the actual regulatory control of sulfate uptake at the root cellular level. The majority of plant cells, including root cells, have the capacity to reduce and assimilate sulfate in their plastids, presumably facilitating local signaling of sulfate uptake as well as distribution and reduc-

tion/assimilation at the cellular level; it is difficult to distinguish local signaling at a cellular level from that at an integrated tissue level, *viz*. shoot to root interactions. It need to be evaluated to what extent H_2S might function in plants, as the first product of the sulfate reduction pathway (Figure 5), as an endogenous gaseous transmitter in the cross-talk between the sulfate reduction pathway in the chloroplasts/plastids and the transcription of sulfate transporters/sulfate reducing enzymes in the nucleus. At the cellular pH, H_2S is largely undissociated and in this form it may easily pass through the membranes. At a whole plant level, it has been shown that H_2S exposure may diminish the activity of sulfate reduction in the shoot by a down-regulation of the expression and activity of APS reductase, the key-regulating enzyme in the sulfate reduction pathway, and it may result in a down-regulation of the expression of sulfate transporters in the shoot.

Impact of enhanced copper levels and sulfur nutrition on the uptake and metabolism of sulfate in Chinese cabbage (*Brassica pekinensis*)

M. Shahbaz, C.E.E. Stuiver, F.S. Posthumus & L.J. De Kok

The uptake, distribution and assimilation of sulfur are modulated by the plant sulfur status and the sulfur demand for growth. Sulfate deprivation of Chinese cabbage resulted in a rapidly induced expression of Sultr1;1 and an enhanced expression of the constitutively expressed Sultr1;2 in the root, accompanied with an increased sulfate uptake capacity. Exposure of Chinese cabbage (*Brassica pekinensis*) to enhanced Cu^{2+} levels ($\leq 5\ \mu M$), resulted in a decreased plant biomass production, an altered mineral composition, an enhanced expression of the Group 1 high affinity sulfate transporters and an enhanced sulfate uptake activity. The up-regulation of the sulfate transporters in Chinese cabbage upon Cu^{2+} exposure was likely not only due to a higher sulfur demand necessary for the synthesis of metal-binding compounds (*viz*. phytochelatins), but might also be the consequence a direct interference/reaction of Cu with the signal transduction pathway involved in the regulation of the sulfate transporters.

Exposure of Chinese cabbage to 4 μM Cu^{2+} resulted in a decreased plant biomass production, an increased dry matter content and an increased shoot to root ratio (Figure 7). Root growth was more rapidly affected than shoot growth, resulting into an increased shoot to root ratio already after 2 days (Figure 7). Sulfate deprivation of Chinese cabbage resulted in a decreased plant biomass production and in a change in shoot to root biomass partitioning in favor of that of the root, as illustrated by a decrease in shoot to root ratio (Figure 7). A simultaneous exposure of plants to sulfate-deprivation and 4 μM Cu^{2+} resulted in an almost similar decrease of plant biomass production as observed upon Cu^{2+} exposure to sulfate-sufficient plants. The shoot to root biomass partitioning, however, was quite similar to that observed for Cu^{2+}-exposed sulfate-sufficient plants (Figure 7). There was an increase in dry matter content of the root of the Cu^{2+}-exposed sulfate-deprived plants after 3 days, whereas that of the shoot remained unaffected. Evidently, upon a 4 day-

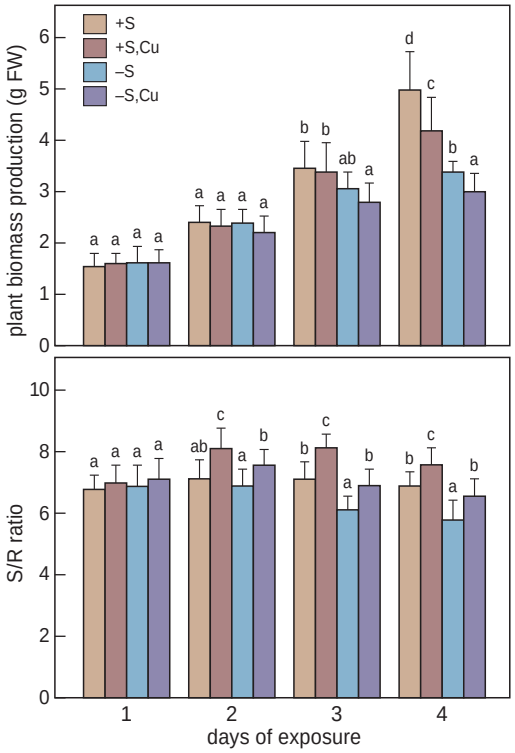

Figure 7. Impact of Cu^{2+} and sulfate deprivation on plant biomass production (g FW) and shoot to root ratio of Chinese cabbage. Seedlings were grown on 25% Hoagland nutrient solution containing 0.5 mM sulfate in a climate-controlled room for 7 days, transferred to a fresh nutrient solution at 0.5 mM sulfate (+S) or 0 mM sulfate (-S) and containing supplemental 0 or 4 μM Cu^{2+} for 1, 2, 3 and 4 days. See for methods (Koralewska *et al.* 2009, Shahbaz *et al.* 2010). Data represent the mean of 15 measurements with 3 plants in each (±SD). Different letters indicate significant differences between treatments within the day of exposure ($p < 0.01$, Student's t-test).

exposure, the impact of sulfate deprivation on plant biomass production was more pronounced (more rapidly) than that at enhanced Cu^{2+} levels.

Cu^{2+} exposure disturbed the sulfate metabolism very rapidly and at sulfate-sufficient conditions, Cu^{2+} exposure resulted in a rapid increase in water-soluble non-protein thiol content in both root and shoot. However, the thiol accumulation was much more pronounced in the root than in the shoot; after 4 days its content had increased 4-fold and 1.5-fold in root and shoot, respectively (Figure 8). The sulfate content of the root was hardly affected upon exposure Cu^{2+}, but its content in the shoot started to increase after 2 days up to 1.5-fold after 4 days of exposure (Figure 8). In contrast, the nitrate content decreased in both root and shoot upon Cu^{2+} exposure (Figure 8).

Upon sulfate deprivation the thiol and sulfate content were strongly decreased in both root and shoot. However, nitrate content of the shoot was increased, whereas that of the root was not affected upon sulfate deprivation (Figure 8). The thiol content in the root of plants simultaneously exposed to sulfate-deprivation and 4 μM Cu^{2+} was increased after 1 day, thereafter it remained unaltered (Figure 8). In the shoot, however, the thiol content was increased after 1 day and remained higher than that of the sulfate plants up to 4 days of exposure (Figure 8). The strong decrease in thiol content in both root and shoot upon sulfate deprivation was most likely due to growth dilution and/or metabolism of thiol compounds to support the synthesis of other essential organic sulfur-containing compounds (e.g. proteins). The thiol content of plants simultaneously exposed to sulfate deprivation and 4 μM Cu^{2+} remained unaltered/higher in the root and shoot than in sulfate-deprived plants. This might indicate that part of the accumulated thiols upon Cu^{2+} exposure, presumably the phytochelatins fraction could not be re-metabolized. The nitrate content of Chinese cabbage simultaneously exposed to sulfate deprivation and Cu^{2+} was not substantially affected in the both root and shoot (Figure 8).

Cu^{2+} exposure rapidly affected the expression and the activity of the sulfate transporters. Exposure of Chinese cabbage to 4 μM Cu^{2+} resulted in an enhanced expression and activity of the sulfate transporters in the root

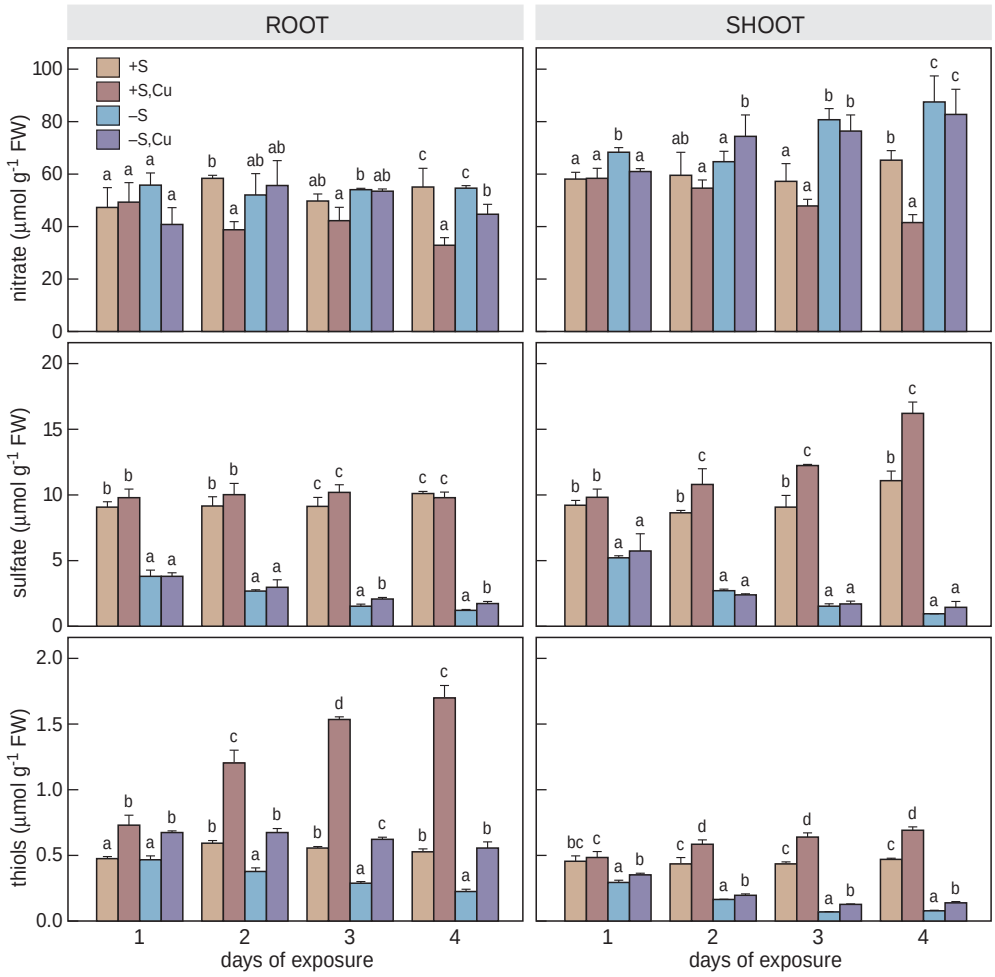

Figure 8. Impact of Cu^{2+} and sulfate deprivation on nitrate, sulfate and thiol content (μmol g^{-1} FW) of Chinese cabbage. For experimental details see legend Figure 1. Data represent the mean of 3 measurements with 3 plants in each (±SD). Different letters indicate significant differences between treatments ($p < 0.01$, Student's t-test).

(Figure 9 en 10). The expression of the constitutively abundant sulfate transporter Sultr1;2 and the sulfate uptake capacity were already up-regulated after 1 day of exposure (Figure 9 en 10). In both root and shoot, also the Group 4 sulfate transporter Sultr4;1 was already up-regulated upon 1 day of Cu^{2+} exposure, whereas in the root also Sultr4;2 was slightly up-regulated upon 4 day of exposure (Figure 9). The expression of APR, the key regulating enzyme in sulfate reduction pathway, was first up-regulated upon 1 day of Cu^{2+} exposure in the root, but after 2 days it was down-regulated again to a level similar to that of non-exposed control plants (Figure 10). The expression APR in the shoot, however, was not affected upon Cu^{2+} exposure (Figure 10). The transient up-regulation of APR in the root may illustrate a temporary up-regulation of the sulfate reduction pathway possibly needed for a Cu-induced synthesis of thiols (presumably glutathione and for a lesser part phytochelatins; Shahbaz *et al.* 2010), in response to excess of Cu taken up by the root

(Figure 9 en 10). Sulfate deprivation also resulted in an enhanced expression Sultr1;1 and Sultr1;2 in the root, an enhanced expression of Sultr2;2, Sultr4;1, Sultr4;2 and APR in both root and shoot, and in an increased sulfate uptake capacity of the root (Figure 9 en 10). A simultaneous exposure of plants to sulfate deprivation and 4 μM Cu^{2+} hardly affected the observed up-regulated expression of the sulfate transporters and APR, but after 2 days it resulted in a slightly less up-regulation of Sultr1;1 in the root along with the decrease in the sulfate deprivation-induced up-regulation of the sulfate uptake capacity of the root (Figure 9 en 10).

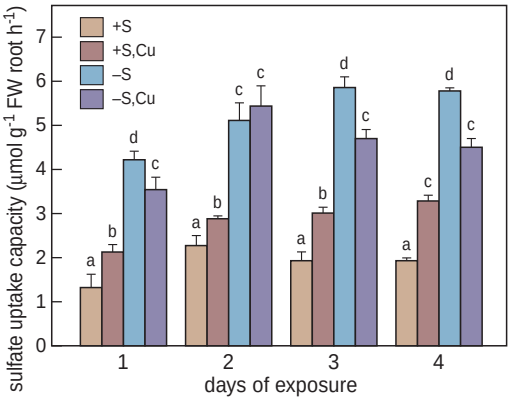

Figure 9. Impact of Cu^{2+} and sulfate deprivation on sulfate uptake capacity (μmol g^{-1} FW root h^{-1}) of Chinese cabbage. For experimental details see legend Figure 1. Data represent the mean of 3 measurements with 3 plants in each (± SD). Different letters indicates significant differences between treatments ($p < 0.01$, Student's t-test).

It is unlikely that the up-regulation of the sulfate uptake could solely be attributed to a higher sulfur need upon Cu^{2+} exposure, since Cu^{2+} exposure also resulted in enhanced sulfate levels in the shoot. The consequences of sulfate-deprivation on plant growth and the expression and activity of the sulfate transporters e.g. the induction and up-regulation of sulfate transporters and APS reductase was hardly further affected by an enhanced Cu^{2+} level, indicating that sulfate deprivation more or less surpassed the development of Cu toxicity.

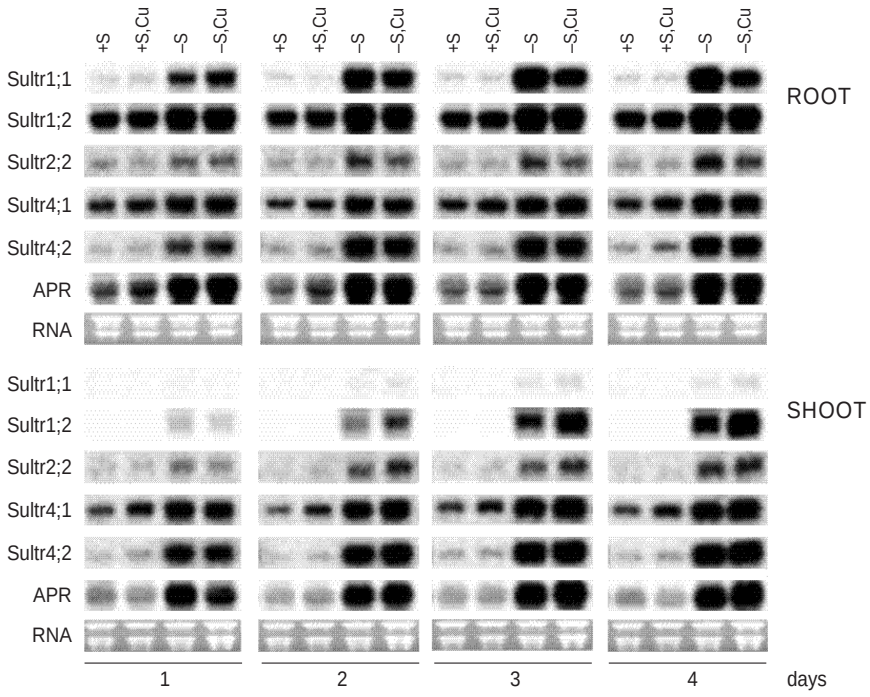

Figure 10. Impact of Cu^{2+} and sulfate deprivation on mRNA abundance of sulfate transporters (Sultr) and APS reductase (APR) in the root and shoot of Chinese cabbage. For experimental details see legend Figure 1.

Interaction between atmospheric and pedopheric sulfur nutrition in *Eucalyptus camaldulensis*

F.S. Posthumus, C.E.E. Stuiver, M. Tausz* & L.J. De Kok

*(Department of Forest and Ecosystem Science, University of Melbourne, Water Street, Creswick, Victoria 3363, Australia)

Plants are able to use foliarly absorbed H_2S as sulfur source for growth, especially under conditions where the sulfur supply to the roots is limited. In *Brassica*, there was direct interaction between atmospheric and pedospheric sulfur utilization and H_2S exposure hardly affected the total plant sulfur content, whereas the expression and activity of the sulfate transporters and APS reductase were reduced. However, in other species *viz. Allium cepa* and *Picea abies*, H_2S exposure hardly affected the uptake of sulfate, whereas it resulted in an accumulation of organic sulfur compounds in the shoot. Apparently, the shoot to root signaling in the regulation of the uptake and assimilation of sulfate differs between species.

Eucalyptus was also able to utilize atmospheric H_2S as sulfur source, resulting in a down-regulation of the sulfate uptake capacity by the root both in the presence and absence of sulfate in the root environment. A 7 day exposure of *Eucalyptus* seedlings to 0.2 μl l^{-1} H_2S at sulfate-sufficient conditions (+S, H_2S) did not affect shoot and root biomass production and shoot pigment content,

Table 2. Impact of a H2S exposure and sulfate deprivation on biomass and pigment content of *E. camaldulensis*. Plants were exposed for 7 days and data on biomass production and dry matter content (DMC) represent the mean of 18 and 6 measurements with 3 plants in each (± SD), respectively. The initial shoot and root weight was 0.14 ± 0.01 g and 0.13 ± 0.02 g, respectively. Data on pigment content represent the mean of 3 measurements and on total nitrogen, total sulfur, nitrate and sulfate the mean of 3 measurements with 3 plants in each (± SD), respectively.

	+S	+S, H_2S	-S	-S, H_2S
SHOOT				
Biomass production (g)	0.25 ± 0.08a	0.18 ± 0.09a	0.28 ± 0.08a	0.22 ± 0.06a
DMC (%)	24 ± 4a	19 ± 4a	22 ± 2a	19 ± 1a
Chlorophyll a+b (mg g^{-1} FW)	1.76 ± 0.04a	1.72 ± 0.07a	1.76 ± 0.05a	1.69 ± 0.60a
Chlorophyll a/b	3.22 ± 0.33a	3.28 ± 0.06a	3.49 ± 0.05a	3.30 ± 0.20a
Total N (mmol g^{-1} DW)	1.43 ± 0.21a	2.29 ± 0.01b	1.10 ± 0.12a	2.15 ± 0.08b
Total S (μmol g^{-1} DW)	74 ± 9a	151 ± 17b	45 ± 19 a	141 ± 11b
Nitrate (μmol g^{-1} FW)	10.2 ± 1.7a	10.2 ± 1.3a	19.0 ± 11.5a	11.2 ± 1.2a
Sulfate (μmol g^{-1} FW)	7.5 ± 0.5b	11.3 ± 1.1d	3.3 ± 0.9a	9.1 ± 1.4c
ROOT				
Biomass production (g)	0.23 ± 0.06a	0.17 ± 0.08a	0.27 ± 0.06a	0.22 ± 0.06a
Root DMC (%)	9.0 ± 0.5a	9.1 ± 0.6a	9.6 ± 1.1a	8.8 ± 0.8a
Total N (mmol g^{-1} DW)	3.05 ± 0.06a	2.83 ± 0.13a	2.47 ± 0.30a	3.01 ± 0.13a
Total S (μmol g^{-1} DW)	114 ± 8b	130 ± 12a	38 ± 12 b	104 ± 6b
Nitrate (μmol g^{-1} FW)	31.8 ± 1.8a	32.2 ± 5.8a	27.8 ± 1.0a	28.0 ± 2.4a
Sulfate (μmol g^{-1} FW)	7.9 ± 0.8d	6.4 ± 1.0c	2.5 ± 1.5a	5.0 ± 0.6b

however it resulted in an increase in total sulfur and sulfate content in both root and shoot and total nitrogen in the shoot (Table 2). The sulfate uptake capacity of sulfate-sufficient (+S) plants was partly down-regulated (by 67%) upon H_2S exposure (+S, H_2S), indicating that plants had partially transferred from sulfate taken up by the root to H_2S taken by the shoot as sulfur source for growth (Figure 11). Sulfate deprivation (-S) resulted in a more than 6-fold increase of the sulfate uptake capacity (Figure 11). If sulfate-deprived plants were simultaneously exposed to H_2S (-S, H_2S), then the sulfate uptake capacity was not up-regulated, but it was even lower than that of sulfate-sufficient non-exposed (+S) plants (Figure 11). For all treatments there was a direct relation between the sulfate concentration in the root and the sulfate uptake capacity (Figure 12). H_2S exposure of sulfate-deprived plants (-S, H_2S) did neither affect plant biomass, nor shoot to root ratio and pigment content, but there was a substantial increase in total sulfur and sulfate content in both root and shoot (Table 2), indicating the absence of a strict regulatory control between shoot and root in atmospheric H_2S and pedospheric sulfate utilization. Moreover, H_2S exposure also resulted in enhanced nitrogen content in the shoot, which could be (similar to that of sulfur) ascribed to an enrichment of the organic nitrogen fraction. The nature of both the enhancement of organic S and N accumulation upon H_2S exposure needs further to be investigated.

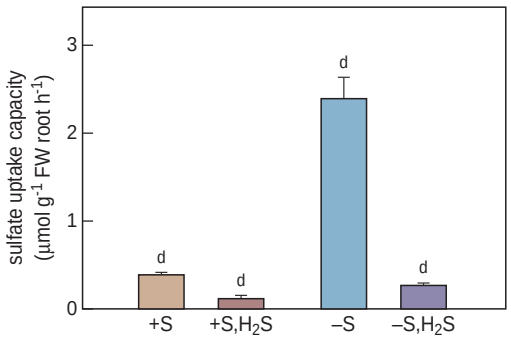

Figure 11. Effect of H_2S exposure and sulfate deprivation on the sulfate uptake capacity of *E. camaldulensis*. Plants were exposed for 7 days and data represent the mean of 3 measurements with 3 plants in each (± SD).

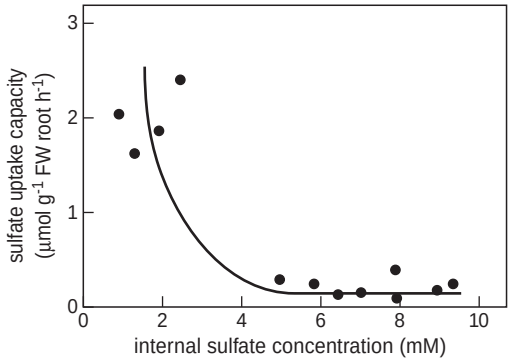

Figure 12. The relation between the sulfate uptake capacity and the overall sulfate concentration in roots of *E. camaldulensis* at various levels of atmospheric and pedospheric sulfur nutrition. Derived from data presented in Table 1.

Selection and Breeding of Robust Rootstocks as Tool to Improve Nutrient-Use Efficiency and Abiotic Stress Tolerance in Tomato

Jan Henk Venema, Leen A. Villerius, Harro J, Bouwmeester* and J. Theo M. Elzenga

*(Laboratory of Plant Physiology, Wageningen University)

In the Dutch glasshouse industry, growers rely on large inputs of mineral nitrogen (N), phosphorus (P) and potassium (K) fertilisers to realize high physical production levels and product quality to produce a profitable crop. By comparison, the annual application of fertilisers is on average about ten times higher for glasshouse than for open field crops. Glasshouse horticulture offers possibilities for full process control; nevertheless it is still faced by low fertiliser efficiency. Therefore, in areas with a high density of glasshouses, the discharges of N and P contribute significantly to groundwater and surface water pollution.

In 1997, the Dutch government introduced legislation aimed at reducing N and P emissions by 95% each by 2010, using 1985 as the year of reference (GlaMi, 1997). Since then, the glasshouse industry has implemented crop specific limitations in N and P use by switching from open to closed substrate culture systems and re-use of drainage water. In practice, substantial loss of water and minerals due to leakage and partial flushing to reduce accumulation of ions still occurs in closed growing systems. The nutrient-use efficiency of closed growing systems for both N and P is currently only 80%. Of the glasshouse-grown tomatoes, 10% are not grown hydroponically, but soil-grown. For soil-grown crops, systems with irrigation and fertilisation strategies based on crop demand and soil moisture content, like the fertigation model, have the best prospective for sustainability. For all growing systems, further improvements are possible by reduction of N and P target values for the root environment. To stimulate realization of the above mentioned targets, the Dutch Ministry of Economic affairs, Agriculture and Innovation (EL&I) has launched a new research program

Figure 13. Schematic representation of the idealized beneficial effects of grafting a modern high-yielding (elite) tomato cultivar onto a vigorous rootstock as tool to improve nutrient-use efficiency and the adaptability to changing growing conditions. Left illustrates an ungrafted elite tomato cultivar and right the same cultivar grafted as a scion onto a vigorous rootstock.
Modified from an original UA-CEAC image created by K. Tomlinson.

"Green Breeding" (2010-2020) with a total of 10 M€ to support plant breeding for sustainable, low-input agriculture and horticulture. The focus is on research for pest and disease resistance breeding, with emphasis on insect resistance, and on improving adaptation to lower input of nutrients and changing climatic conditions. The results should be applicable in and serve both organic and conventional agriculture and horticulture. Both active and significant financial participation of breeding companies is one of the preconditions. In February 2010, four projects were granted for the first phase (2010–2013) including our tomato rootstock project.

A special technique of adapting horticultural crops to counteract environmental stresses is grafting elite, commercial cultivars onto selected vigorous rootstocks. With grafting, the best of two worlds can be combined: excellent above-ground traits (fruit quality and yield), conferred by an elite cultivar used as scion, and desired under-ground traits (resistance against soil-borne diseases and vigorous root growth), conferred by a suitable rootstock (Figure 13). Grafting is nowadays regarded as a rapid alternative tool to the relatively slow breeding methodology aimed at increasing environmental-stress tolerance of fruit vegetables. This project explores the pos-

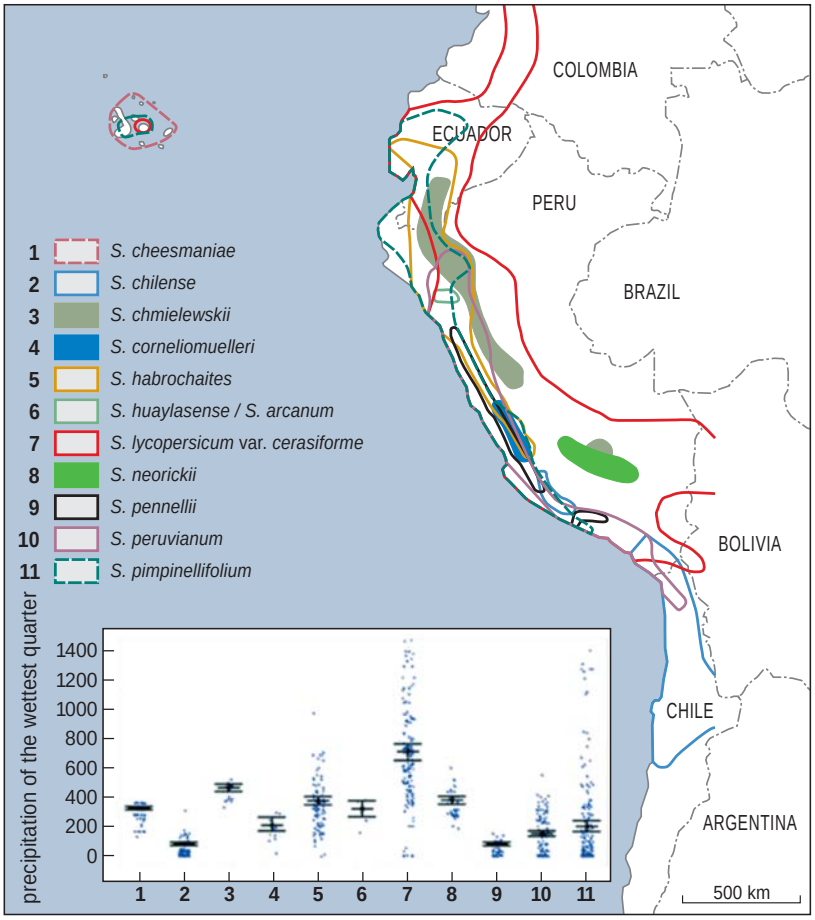

Figure 14. Geographical distribution of wild tomato species in western South-America. The environmental gradients in precipitation, temperature and salinity within this geographical range is large and very interesting to select accessions with improved abiotic stress tolerance. Taken from Boyle, LC (2008), Evolution 62: 2995-3013.

sibilities of using rootstocks as a tool to improve the nutrient-use efficiency and abiotic stress tolerance of elite tomato cultivars. In addition, it aims to deliver key-physiological parameters (biomarkers) which can be used for the selection and breeding of rootstocks which improve the sustainability and profitability of both the conventional and organic cultivation of tomatoes. So far, the breeding for vigorous rootstocks is a matter of trial and error since practical selection tools like genetic markers are lacking. This is because the knowledge about the physiology behind a successful rootstock (root-shoot interaction) is still very limited. In addition, desired root traits studied in this project, nutrient-uptake efficiency, salt and suboptimal-temperature tolerance, are complex and the identified quantitative trait loci (QTL's) have hardly any value for practical breeding purposes. This project will therefore not focus on the identification of single allele-specific markers for traits that are expressed in a particular hybrid combination. Instead, it aims to identify biomarkers (particular physiological traits) as generic tools to develop a screening method to select robust and vigorous rootstocks that improve the nutrient-use efficiency and adaptability of tomato to new growing, climatic and environmental conditions in open and protected cultivation. If successful, this tomato rootstock project will contribute to the improvement of the sustainability and profitability of both conventional and organic cultivation of tomatoes.

Since the genetic variation in the cultivated tomato (*S. lycopersicum*) is very limited, the project started this year with the selection of 10 out of 27 related wild tomato accessions to broaden the variance in abiotic stress tolerance and nutrient-use efficiency (Figure 14). The breeding companies involved in this project are currently creating hybrids between elite tomato cultivars and the selected wild tomato accessions. In the next three years of this project these F1's will be used as rootstock to elucidate the key-physiological traits which underpin desired root(stock) traits.

Comparison of The Na^+/H^+ antiport activity in *Beta vulgaris* and *Zea mays vacuoles* by the use of on selective microelectrode

Fatma Z.M. Ali and J. Theo. M. Elzenga

Plant cells maintain a K^+/Na^+ ratio in their cytosol with relatively high K^+ (100–200 mM), and low Na^+ (1–10 mM) concentrations1. Thus, the efficient exclusion of Na^+ from the cytoplasm by transporting it into the vacuole is one of the main mechanisms for the adaptation of plants to salt stress. Accumulation of Na^+ in the vacuole is typically carried out by a secondary transporter known as Na^+/H^+ antiporter, which is driven by the proton-motive force generated by the H^+-ATPase and H^+-pyrophosphatase in the tonoplast. As we already mentioned in the CEES 2009 report, a new technique is now available to measure ion fluxes from the surface of single cells and vacuoles by the use of the MIFE (Microelectrode ion flux estimation) system. The developed method was used to measure the activity of the vacuolar Na^+/H^+ antiport for the first time by the use of the Microelectrode Ion Flux Estimation (MIFE) system, a variant of the vibrating microelectrode technique.

The researcher (Fatma Zaki) holding the salt tolerant maize variety (Arizona 8601) grown hydroponically under 50 mM NaCl concentration. The vacuolar Na^+/H^+ antiport activity of Arizona 8601 allow it to tolerate salt concentrations considered lethal for other maize cultivars.

Previously, the Na^+/H^+ antiport activity was measured using the acridine orange quenching method which our lab worked on one important publication1 for that technique and was among the most cited papers for the Na^+/H^+ antiport activity studies. However, application of the acridine orange fluorescence quenching method requires a time consuming preparation of purified tonoplast vesicles from several grams of plant material. This limits its application only to tissue of which large quantities of homogeneous tissue is available, like sugar beet storage tissue, or when tissue- or cell type-specificity of the antiport activity is not relevant. Since these measurements are performed on tonoplast vesicles the preparation will contain vesicles of both the inside-out and inside-in orientation in an unknown proportion, limiting the resolution even in comparative studies. We propose that by use of the vibrating microelectrode technique some of these disadvantages of the acridine orange technique can be avoided. The technique depends on the isolation of protoplasts and the subsequent osmotic release of the vacuole. This can be a fairly quick procedure and allows measurements on individual vacuoles of specific cell types and with a known, outside-out, membrane orientation.

Changes induced by the addition of NaCl to the bath medium in both pH and the proton fluxes could be measured on individual vacuoles. In vacuoles isolated from red beet storage tissue, the addition of MgATP to the bath solution induced an influx of proton across tonoplast and an increase in the pH in the unstirred layer (Figure 15). The subsequent addition of NaCl (50 mM) caused a proton efflux and acidification of the bath solution (Figure 15). As the efflux of protons only was observed when NaCl or $NaNo_3$ was added and not when KNo_3 or choline chloride was used the effect seems to be sodium-specific. The Na-induced H^+ efflux was inhibited by amiloride, a Na^+/H^+ antiport inhibitor. Two salt tolerant maize cultivars, SR15 and Arizona 8601, were tested for the presence of Na-induced H^+ efflux activity. Evidence was found for the presence of Na^+/H^+ antiport in tonoplast of the root cortical cells of cultivar Arizona 8601 as the addition of 50 mM NaCl resulted in a H^+ efflux comparable to the fluxes observed in red beet vacuoles. Na^+/H^+ antiport activity was not detected in vacuoles isolated from root cells of cultivar SR15 (Figure 16).

Our results show that the MIFE system is sensitive enough to study ion fluxes, likely

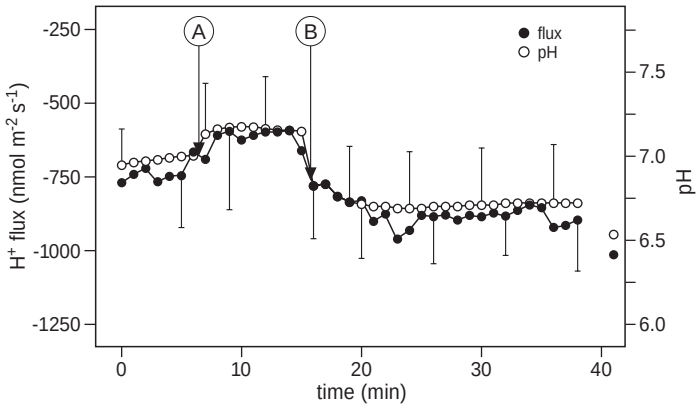

Figure 15. Changes in pH and proton fluxes from beet root vacuoles (n = 3) by the addition of A: 1 mM MgCl2 and B: 50 mM NaCl.

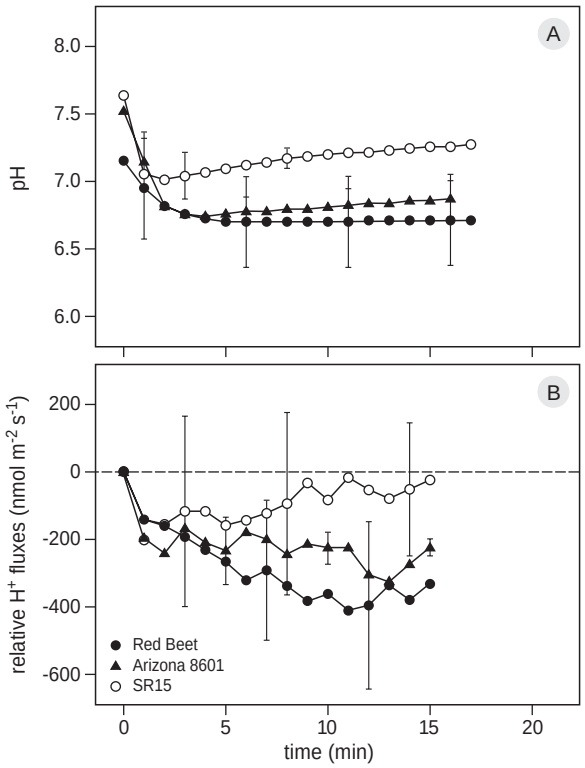

Figure 16. Effect of NaCl (50 mM) addition on A: the pH and B: relative proton fluxes from maize and red beet vacuoles (n ≥ 3). Y = 0 represents the values before the addition of the salt

carried by antiporters across the tonoplast of root cells. The sensitivity and time resolution of the ion-sensitive microelectrode vibrating probe technique can allow further study on different membrane transporters. In the Future, the study of vacuolar chloride channels and other anion porters with the MIFE technique will be of considerable interest when trying to elucidate the contribution of Cl- to salt stress. In principle, using the vibrating microelectrodes to study ion channels and transporters in an individual vacuole, i.e. using Arabidopsis knock-out mutants, would be a valuable complement to patch clamp studies on specific channels.

Characterizing genes regulating programmed cell death in the model plant species *Arabidopsis thaliana*

Sujeeth Neerakkal; Muhammad K Qureshi; Nikolay Mehterov; Jacques Hille and Tsanko S. Gechev

Plant cells possess unique organelles such as chloroplasts and vacuoles, which play central roles in processes such as oxidative stress tolerance, senescence and cell death. At the molecular level, plant specific genes and transcription factors are involved in reactive oxygen species metabolism, stress tolerance and programmed cell death (PCD). Genetic and molecular biological studies have suggested that genes which modulate oxidative burst/ PCD are much more than those implicated so far.

Paraquat and aminotriazole are two herbicides that trigger accumulation of superoxide radicals and hydrogen peroxide, respectively, during oxidative burst induced cell death. Using genetic screens we have isolated new plant mutants with enhanced tolerance to paraquat and aminotriazole. Genetic analysis and map-based cloning are used to identify the mutated genes responsible for the enhanced tolerance. Unraveling the genetic mechanisms of oxidative stress tolerance/PCD is not only of fundamental value but also of significant practical importance for generating crops with improved qualities.

Editorial activities

Plant Biology (Wiley-Blackwell)
Editors in chief: H. Rennenberg and J.T.M. Elzenga
Print ISSN: 1435-8603 Online ISSN: 1438-8677
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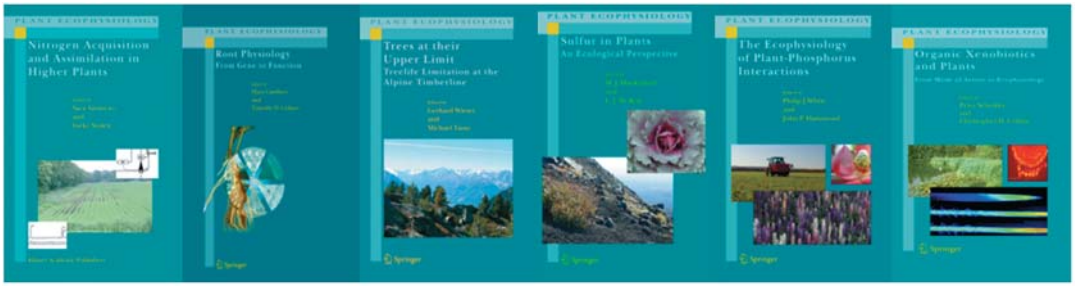
Plant Biology is an international journal of broad scope bringing together different subdisciplines, such as physiology, molecular biology, cell biology, development, genetics, systematics, ecology, evolution, ecophysiology, plant-microbe interactions, and mycology. Plant Biology publishes original problem-oriented full-length research papers, short research papers, and review articles. Discussion of hot topics and provocative opinion articles are published under the heading Acute Views. From a multidisciplinary perspective, Plant Biology will provide a platform for publication, information and debate, encompassing all areas, which fall within the scope of plant science. Plant Biology is the official journal of the German Botanical Society and the Royal Botanical Society of the Netherlands.
In 2010, five regular issues were published and two Special Issue: “Poplar Molecular Physiology” and “Dynamics and Regulation of Plant Membrane Transport”.



Plant Ecophysiology (Springer)
Series Editors: Luit J. De Kok, Malcolm J. Hawkesford and Ineke Stulen
ISSN: 1572-5561; www.springer.com/series/6193

The Springer Series Plant Ecophysiology comprises a series of volumes that deal with the impact of biotic and abiotic factors on plant functioning and physiological adaptation to the environment. The aim of the Plant Ecophysiology series is to review and integrate the present knowledge on the impact of the environment on plant functioning and adaptation at various levels: from the molecular, biochemical and physiological to a whole plant level. This series is of interest to scientists who like to be informed of new developments and insights in plant ecophysiology, and can be used as advanced textbooks for biology students.

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4. Lambers, H. and Colmer, T.D. (eds.) 2005. Root Physiology. From Gene to Function. Springer, ISBN 978-1-4020-4098-9
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Organisation of international meetings

8th International Workshop on Sulfur Metabolism in Higher Plants entitled "Sulfur Metabolism in Plants: Mechanisms and Application to Food Security and Responses to Climate Change" held at the University of Melbourne - Creswick Campus, Water Street, Creswick, Victoria 3363, Australia, November 22-27, 2010. This workshop was jointly organized by the University of Groningen (L.J. De Kok, Chairman) and the University of Melbourne (M. Tausz, Secretary).

5th International Symposium on DMS(P) and Related Compounds (Goa, India, Oct 19-22, 2010). J. Stefels was member of the Scientific Committee.

Publications 2010

Doctorate granted by the institution, prepared within the institution

C. Schmitz 2010. The cost of living. Temperature compensation of the metabolic rate in plants. Promotor: prof. dr. J.T.M. Elzenga. Rijksuniversiteit Groningen. ISBN: ISBN: 978-90-367-4558-1; 978-90-367-4559 (digital version).

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Evolutionary Genetics

Group leader Prof. dr. L.W. Beukeboom

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Dr. L.P.W.G.M. van de Zande	RUG	1.0	
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Dr. J.G. de Boer	NWO-ALW VENI	0.8	071001-101001
Dr. B.A. Pannebakker	NWO-ALW VENI, RUG	1.0	080915-110915
Dr. T. Schwander	NWO-ALW VENI	1.0	100201-130201
Dr. C.J. Vermeulen	RUG	1.0	090815-100815
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Drs. W. Diao	RUG-Marie Curie	1.0	090101-130101
Drs. S. Gerritsma	RUG	1.0	090706-130706
Drs. E. Geuverink	NWO-TOP	1.0	100901-140831
Drs. M.C.W.G. Giesbers	NWO-ALW	1.0	080901-120831
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Drs. T. Koevoets	NWO-ALW	1.0	060901-100901
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Prof. dr. M. Schilthuizen	Naturalis		

Introduction

The *Evolutionary Genetics Group* investigates genetic and life-history traits of organisms from an evolutionary perspective. We ask how population structure and genetic variation affect and, in turn, are shaped by, evolutionary and ecological processes. Research focus is on the genetics and evolution of reproductive systems and life histories, genetics of speciation, genetics and genomics of host-parasitoid interactions, and the genetics and evolutionary ecology of biodiversity. We put special emphasis on the integration of theoretical approaches and experimental testing of models.
Our research is aimed at unravelling the genetic regulation of reproductive systems and at understanding how reproductive modes shape mating systems and life-history characteristics. The main focus is on haplodiploidy in insects, which combines parthenogenetic and sexual reproduction, as well as differences in ploidy level of males and females. We also study genomic, genetic and ecological adaptations in parasite host-interactions. Our molecular approach includes the development of molecular markers for population structure analysis and genome mapping, the identification and structural analysis of genes and their regulatory regions and expression profiling. Our study organisms are parasitoid wasps (*Nasonia, Asobara, Cotesia*), flies (*Drosophila, Musca*) and stick insects (*Timema*).

Our main research themes are: (1) Genetics and evolution of sex determining mechanisms; (2) Genetics and evolution of reproductive isolation; (3) Genetics, ecology and evolution of life-history traits and reproductive strategies; (4) Genomics of host-parasitoid interactions; and (5) Evolutionary and ecological drivers of biodiversity. Below we present our highlights, as well as several projects that showcase the diversity of our research.

Highlights of Evolutionary Genetics

2010 was an absolute top year for Evolutionary Genetics. It all started in January with seven publications within one month. One of these was the official publication of the Nasonia Genome Project in *Science* (Werren *et al.* 2010). During the international *Drosophila* conference in Washington in 2004 the idea was raised to sequence the complete genome of our model organism, the parasitic wasp *Nasonia*. As a follow up we wrote the required White Paper (official proposal) with American colleagues at a meeting in Groningen. After approval by the National Institute of Health

Figure 1. Newsfocus in Science upon the 2010 publication of the sequenced and annotated *Nasonia* genome in Science by the *Nasonia* Genome Working Group of which Evolutionary Genetics was one of the founding members.

(NIH), the Baylor College of Medicine in Houston performed the actual sequencing work. An international consortium of scientists subsequently annotated the genome which resulted in the publication in *Science* in January 2010 (Figure 1). Simultaneously with this publication two special editions appeared on *Nasonia* research, one in *Heredity* and one in *Insect Molecular Biology*. Our group participated in four publications in these special issues.

Another 2010 highlight was the elucidation of the sex determination mechanism of *Nasonia*, which was again published in *Science* (Verhulst *et al.* 2010). This was the subject of the PhD project of Eveline Verhulst under supervision of Louis van de Zande. It involved a completely new mechanism of sex determination and meant the solution of a long-standing open question since the middle of the last century. As a follow-up we were invited to write a review on insect sex determination in Current Opinion in Genetics & Development. Later in the year Leo Beukeboom received a TOP grant from the Netherlands Organisation for Scientific Research (NWO) to continue this research.

Other 2010 highlights, in the popular scientific genre, include the publication of an article on the genetics of honeybees by Bart Pannebakker and Kim Meijer in a special issue on honeybees of the Cahiers in Bio-wetenschappen en Maatschappij (Figure 2). Finally, half of our group managed to move in December 2010 to the new Centre for Life Sciences in Zernike.

Figure 2. Cover of 2010 popular scientific publication on honeybee biology. Evolutionary Genetics group members Bart Pannebakker and Kim Meijer contributed a chapter on honeybee genetics and genomics.

Genetics of sex determination in the haplodiploid wasp *Nasonia vitripennis*

Eveline Verhulst, Leo Beukeboom and Louis van de Zande

How gender is determined under haplodiploidy in the absence of heteromorphic sex chromosomes is still an unanswered question, although much progress has been made in recent years. Under haplodiploidy, males and females differ in ploidy level; females are diploid and develop from fertilized eggs, whereas males are haploid and develop parthenogenetically from unfertilized eggs (Figure 3). This mode of reproduction occurs in several invertebrate groups including pinworms, mites, thrips, and beetles, but occurs ubiquitously only in the hymenopteran insects (ants, bees, wasps and sawflies). Sex determination in the haplodiploid honeybee, *Apis mellifera*, depends on the allelic state of a single locus. This mode of sex determination is called complementary sex determination (CSD) and has now been reported for over 60 hymenopteran species. Under CSD, matings between males and females that share a similar *csd* allele results in an increase of diploid homozygous males. Although diploid males are known from *Nasonia*, they do not arise from inbreeding. Various alternative models of sex determination in *Nasonia* have been proposed. In the previous year report, we surmised that sex determination in *Nasonia* may depend on genomic imprinting. Now, we have functionally shown that maternal control is the core of haplodiploid sex determination in *Nasonia* and we present a model how imprinting may be involved in this process.

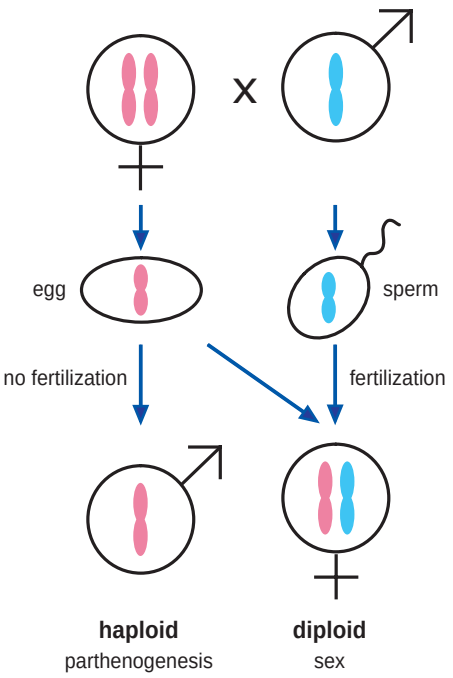

Figure 3. Haplodiploid reproduction.

The gene *transformer* (*tra*), originally identified in *Drosophila melanogaster*, plays a central role in all insect sex determination. We screened the *Nasonia* genome for motifs matching *Drosophila transformer* and *Apis feminizer* (*fem*), an ortholog of *tra*, which resulted in the identification of a single gene. This gene was named *Nasonia vitripennis transformer* (*Nvtra*). Reverse-transcriptase polymerase chain reaction (RT-PCR) showed that transcripts from *Nvtra* are sex-specifically spliced: female-specific splicing retains only the first part of exon two and yields a single transcript encoding a full-length protein. In male *Nasonia*, either the complete exon two or different 3′ parts of exon two can be retained by cryptic 3′ splice-site recognition to yield three different transcripts, all of which encode truncated proteins (Figure 4).

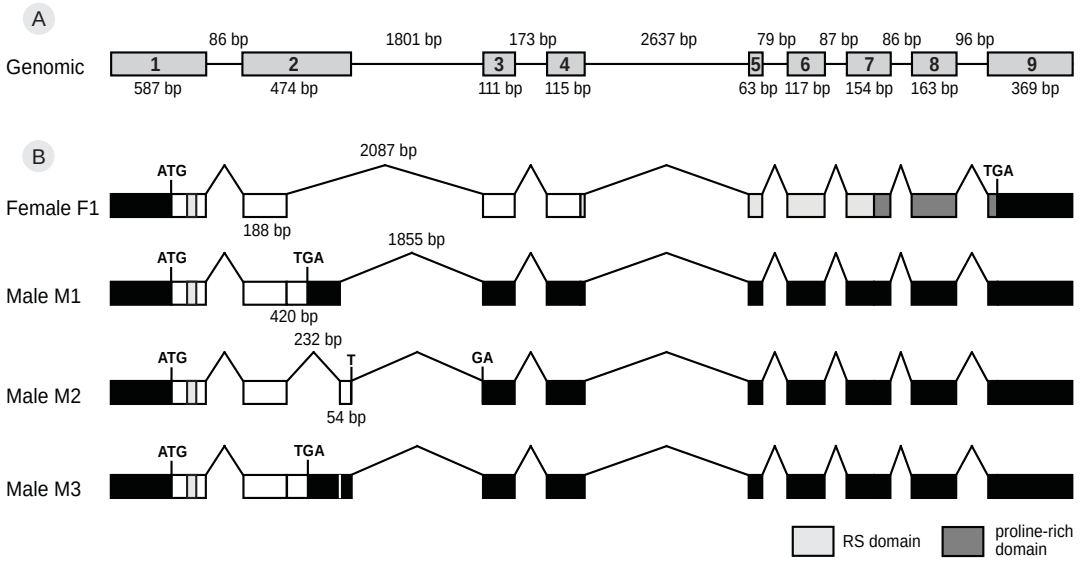

Figure 4. Structure of the *Nvtra* gene. In black: 5' and 3' UTR, white: coding region. In exons 1 and 4–7 RS-rich domains are depicted as light grey. In exon 7–9 a proline rich domain is indicated as dark grey. Female (F1) and male (M1-3) specific splice forms are indicated.

In order to ascertain the role of *Nvtra* in *Nasonia* sex determination, we blocked its function by RNA-interference (RNAi). RNAi knockdown of *Nvtra* in females in the late pupal stage resulted in complete sex reversal of their fertilized eggs, causing them to develop into diploid males rather than females. Interestingly, the *Nvtra* dsRNA-injected females were functionally normal females, but had a decreased amount of female-specific splice form and produced all three male-specific *Nvtra* splice forms (Figure 5). Apparently, repression of *Nvtra* also disrupted female-specific splicing of *Nvtra* pre-mRNA itself. In *Nvtra* dsRNA-injected females, also the expression of the predominant female splice form of *doublesex* (*Nvdsx*) decreased, whereas expression of the male-specific splice form increased (Figure 5). This indicates that, in *Nasonia*, a functional *Nvtra* protein is necessary for female-specific splicing of both *Nvtra* and *Nvdsx* mRNA. The results also indicate that that the essential period of the *Nvtra*/*Nvdsx*-mediated gender determination is before the pupal stage.

Since RNAi treatment of the mothers resulted in the development of diploid males, we assumed that maternal provision of *Nvtra* mRNA to their eggs is an essential part of *Nasonia* sex determination. We monitored the relative levels of *Nvtra* and *Nvdsx* mRNA during early and late embryonic development. In 0- to 1-hour-old embryos, an eightfold excess of *Nvtra* over *Nvdsx* was observed ($t_{(df=18)}$= 3.62, p = 0.0020). Because no appreciable zygotic gene expression occurs at this early stage, this relatively high level of *Nvtra* mRNA must be provided to the egg during oogenesis as a maternal factor and should be the female-specific splice variant only. RT-PCR confirmed this expectation, by showing only female-specific transcripts of *Nvtra* in 0- to 5-hour-old embryos from both fertilized and unfertilized eggs. Our results suggest that a threshold level of maternally provided female-specific *Nvtra* mRNA is essential for

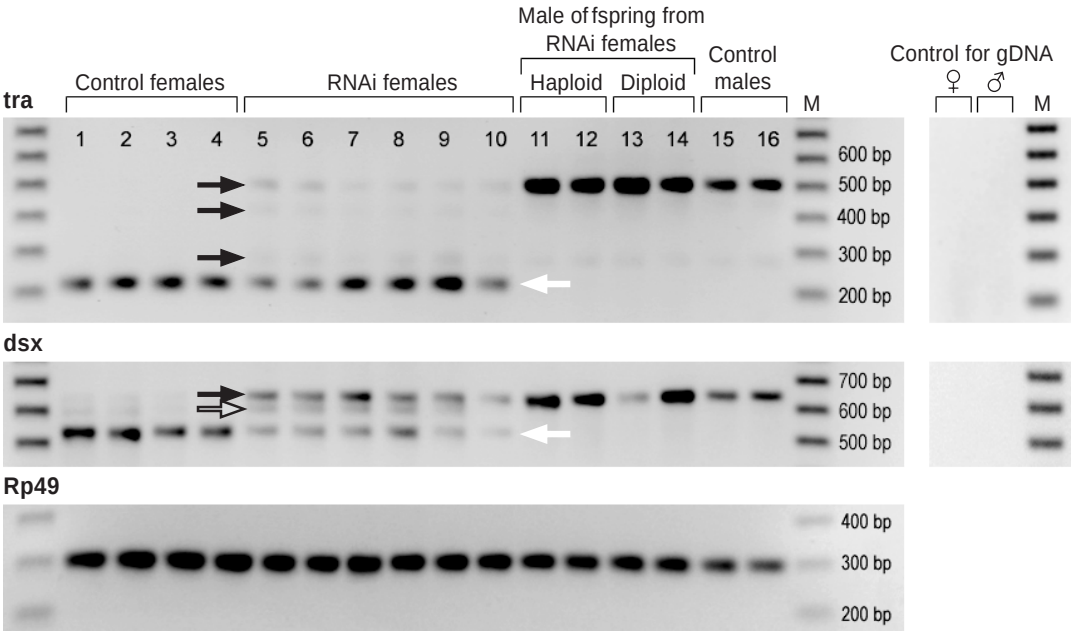

Figure 5. RT-PCR analysis of sex-specific splicing of *Nvtra* (top), *Nvdsx* (middle), and Ribosomal protein 49 (bottom) mRNA. Lanes 1 to 4, control females; lanes 5 to 10, *Nvtra* dsRNA injected females; lanes 11 and 12,haploid male off spring from injected females; lanes 13 and 14, diploid male offspring from injected females; lanes 15 and 16, haploid male offspring from control females. M is a 100-bp molecular size marker. Black arrows indicate male-specific splice forms, gray arrow indicates an unknown splice form, and white arrows indicate female-specific splice forms. A control for amplification from residual genomic DNA is present in the rightmost panel.

female development of the fertilized egg, because knockdown of *Nvtra* in mothers leads to the production of diploid male offspring. They also indicate that female-specific *Nvtra* splicing depends on an autoregulatory loop.

In diploid insect species, the presence of a paternal genome and in the honeybee the hemizygotic or homozygotic state of the *csd* locus is responsible for preventing female specific splicing of *transformer*. Both mechanisms are absent in *Nasonia*, implying that a different mechanism is responsible for the development of males in the presence of maternally provided *Nvtra* mRNA or protein. Because fertilization perse had been ruled out as a sex-determining factor in *Nasonia* before and because

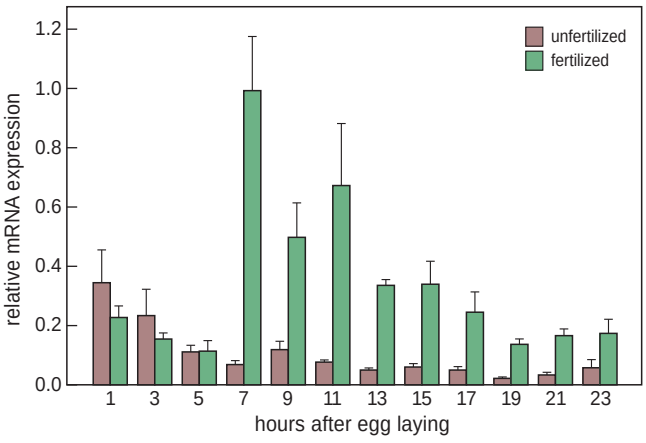

Figure 6. Relative *Nvtra* mRNA levels in embryos from unfertilized and fertilized eggs at different developmental times, indicated as hours after egg laying. Error bars represent SE.

unfertilized eggs will develop as males, we asked whether the presence of a paternal genome together with a maternal genome explains why only fertilized eggs develop as females. Quantitative PCR (qPCR) showed that in 1- to 3-hour-old embryos from both fertilized and unfertilized eggs, the maternally provided *Nvtra* mRNA input gradually decayed. In embryos from unfertilized eggs, a low level of *Nvtra* mRNA was maintained throughout the 23 hours of embryonic development. In sharp contrast, a 15-times-higher expression of *Nvtra* in embryos from fertilized eggs was observed 7 hours after egg laying ($t_{(df=8)} = 4.18$, $p = 0.0031$), which cannot be explained by doses differences due to the presence of two versus one *Nvtra* alleles in these embryos and calls for a regulatory explanation. After this peak expression, a significantly higher level ($F_{(df=15,63)} = 5.25$, $p < 0.0001$) of *Nvtra* mRNA was maintained as compared with embryos from unfertilized eggs (Figure 6). This higher level of *Nvtra* mRNA in diploid embryos consisted of the female-specific splice form, whereas the low levels of *Nvtra* in haploid embryos consisted of male-specific splice forms. We hypothesize that the absence of sufficient zygotic *Nvtra* expression to initiate the autoregulatory loop results in default male-specific splicing. However, in embryos from fertilized eggs, the female-specific splicing of *Nvtra* is maintained because of the availability of zygotic *Nvtra* mRNA.

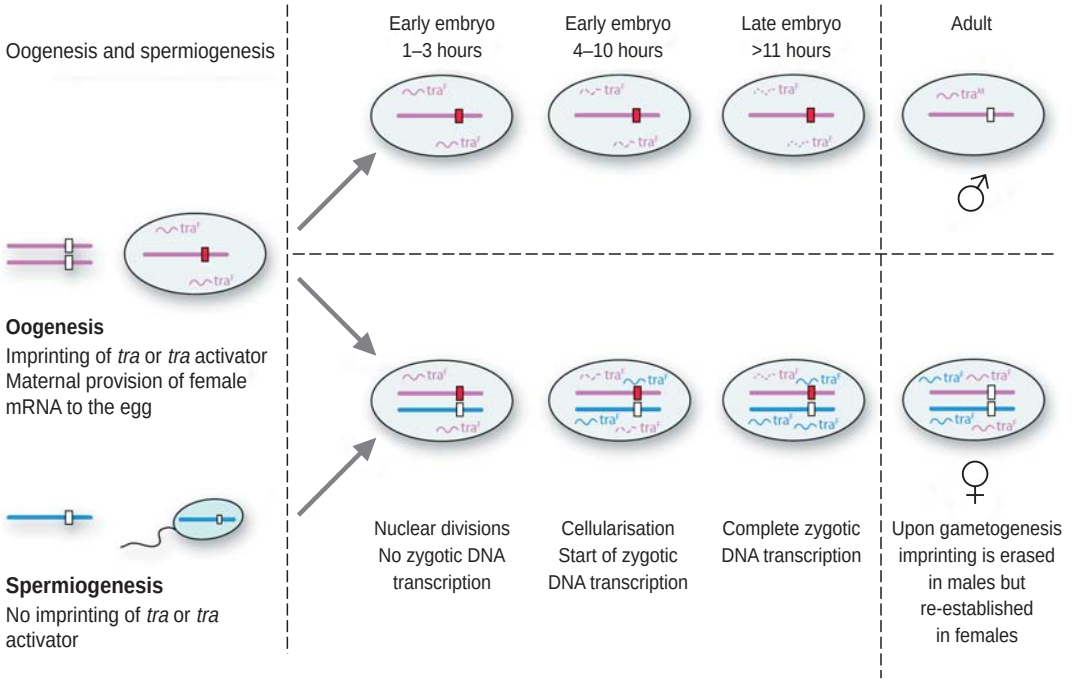

Figure 7. A proposed model for maternal imprinting in *Nasonia* sex determination. Females provide all their eggs with *Nvtra* mRNA. Upper panels: During gametogenesis, the female imprints her genome so that transcription of the *transformer* gene is prevented. Therefore, during development of an unfertilized egg, maternally provided *Nvtra* mRNA (in the female specific splice form) will gradually decay. Any *Nvtra* transcript that will be generated in a later stage will be in the default male splice form, as no autoregulatory loop can initiate. Lower panels: Males do not imprint their genome to prevent zygotic *Nvtra* transcription enabling timely zygotic *Nvtra* transcription to use the maternally provided *Nvtra* mRNA to initiate the autoregulatory loop that ensures female specific splicing of the newly synthesized *Nvtra* transcripts.

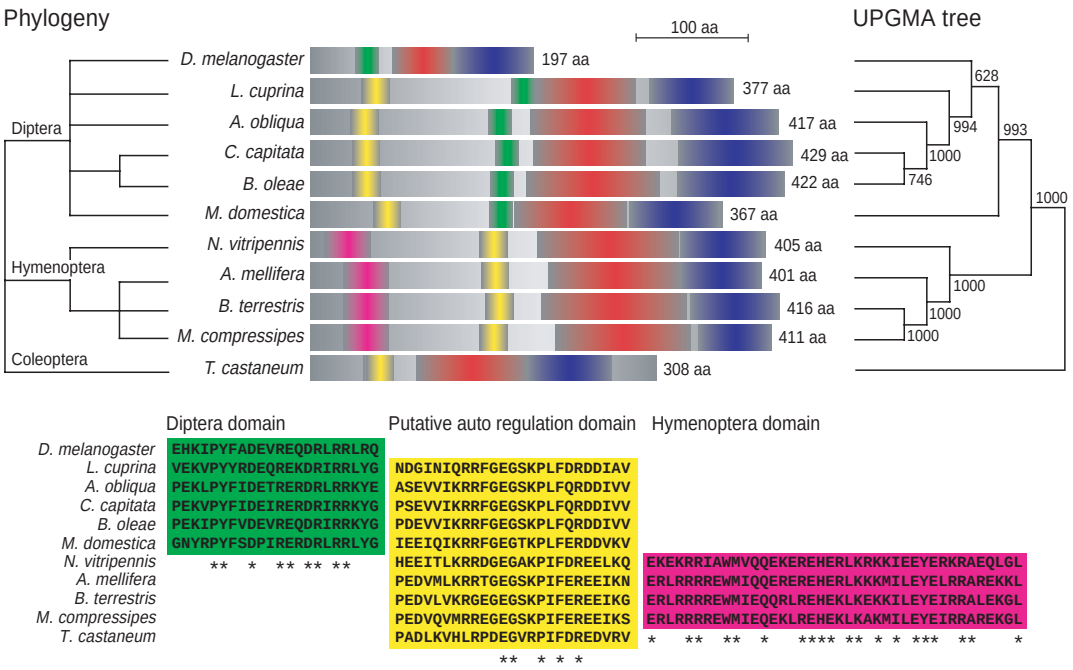

Figure 8. Amino acid alignment and protein sequence tree of tra/fem proteins identified in insect species from three different orders. Upper part: Insect classification on the left is redrawn from Sanchez et al. (2008). On the right is the UPGMA consensus tree of the transformer protein sequences with bootstrap values indicated at the nodes (UPGMA cluster with a Jones-Taylor-Thornton matrix, 1000 bootstraps). In the middle is the protein sequence alignment showing all conserved areas. Green box indicates conserved domain in Diptera, yellow box indicates conserved domain in all species except *D. melanogaster* and purple box indicates conserved domain in Hymenoptera. Red box indicates shared Arg/Ser domains and blue box the common Pro-rich region. Lower part: Alignment of the conserved domains with similar colors as in the complete protein alignment (upper part). aa = amino acids. From top to bottom organisms and GenBank accession no.: *Drosophila melanogaster* (AAF49441); *Lucilia cuprina* (ACS34689); *Anastrepha obliqua* (ABW04165); *Ceratitis capitata* (AAM88673); *Bactrocera oleae* (CAG29243); *Musca domestica* (ACY40709); *Nasonia vitripennis* (NP 001128299); *Apis mellifera* (ABV56235); *Bombus terrestris* (ABY74329); *Melipona compressipes* (ABV79891) and *Tribolium castaneum* (XP 001809947).

Our data show that maternal provision of *Nvtra* to all embryos, followed by sufficient early zygotic *Nvtra* expression, which occurs only in fertilized eggs, is necessary for female development in *Nasonia*. RNAi treatment decreased the maternal provision of *Nvtra* to the eggs, which alone would be sufficient for the production of diploid males. It is possible that the resulting small interfering RNAs (siRNAs) were also transmitted to the eggs, resulting in a decrease in zygotic *Nvtra* transcript expression in addition to a decrease in maternal *Nvtra* input. Either way, the simplest explanation for the mechanism behind *Nasonia* sex determination appears to be maternal input of *Nvtra* mRNA combined with a form of maternal imprinting. A possible model is presented in Figure 7.

Tra regulation of *dsx* apparently constitutes the core of insect sex determination. It likely acquired its function in the early ancestors of the insects, as *tra* orthologs are found throughout the insect class including Diptera, Hymenoptera and Coleoptera, but apparently has no sex determining function in the crustacean Daphnia. The large sequence divergence

indicates that *tra* conservation is predominantly at the functional and less at the structural level. This becomes apparent when TRA protein sequences are compared among species. Comparison of the insect phylogeny to a phylogeny based on the TRA protein sequence reveals that its evolution has followed species divergence confirming that conservation lies in function rather than sequence (Figure 8). Strikingly, an alignment of TRA orthologs shows that only the proline and Arg/Ser rich regions are conserved throughout the examined insect species, reflecting their function as splice factor. One additional domain is conserved in Hymenoptera only, a second domain is conserved in all species except *Drosophila* and a third domain is conserved in all Diptera. The second domain may function in *tra* auto-regulation that is absent in *D. melanogaster* and replaced by *Sexlethal*. The other two domains are apparently not involved in *tra* splicing but may have other unknown functions. A striking example of the central role of *tra* in the evolution of insect sex determination is the complete reversal in the paternal regulation of *tra* upon the separation of Hymenoptera and Diptera.

Genomic characterisation of inbreeding depression

Corneel Vermeulen in collaboration with: Kamilla S Pedersen[1], Hans C Beck[2], Jørgen Petersen[2], Volker Loeschcke[1]

1. Ecology and Genetics, Department of Biological Sciences, Aarhus University, DK-8000 Aarhus, Denmark; 2Danish Technological Institute, DK-6000 Kolding, Denmark

Virtually all genomes are burdened with a number of deleterious mutations, which at the population level contribute to the genetic load. In non-haploid organisms a large portion of the genetic load remains hidden, because many deleterious alleles are only phenotypically expressed in homozygous form. An increase in the level of homozygosity (e.g. through inbreeding) will cause significant discomfort to the afflicted individuals, who will suffer from one to several genetic diseases. This phenomenon, widely known as inbreeding depression, has major relevance for agriculture, medical sciences, conservation biology and of course evolutionary biology.

Although the population genetic principles of inbreeding depression are well known, the functional genomic basis remains poorly characterised. This warrants the detailed study of genetic loci giving rise to inbreeding depression. Since the complex and polygenic nature of general inbreeding depression makes this a formidable task, we focus on one aspect, conditional lethals in the fruitfly model system (D*rosophila melanogaster*) (Figure 9). Highly inbred lines harbouring conditionally expressed lethals display large and reproducible inbreeding effects at restrictive thermal conditions, but are otherwise easy to culture and handle. We use genomic, transcriptomic and proteomic approaches to characterise the inbreeding event and identify the lethal alleles. This will ultimately allow us to assess whether similar inbreeding effects consistently map to the same genomic regions and whether genetic variation at these regions is associated with variation in thermal tolerance in outbred populations.

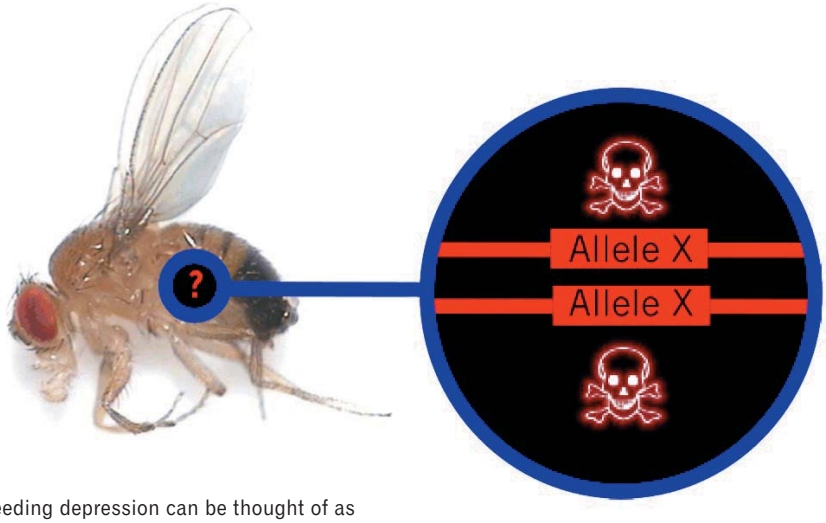

Figure 9. Inbreeding depression can be thought of as the expression of recessive genetic diseases that are lurking in the genome.

Here we present the results of a study on the changes in the complete set of expressed proteins (proteome). We focused on an inbred line displaying severe cold sensitivity (the L-line). The conditional nature of the inbreeding event allowed for an elegant experimental set-up. We used two control lines: the traditional outbred control (OC) line and also an inbred control (IC) line. All lines were exposed to a benign environment (the permissive conditions, yet another control) and to a mild cold shock (the restrictive conditions). The cold shock was applied by submerging culture bottles with flies in an ice bath. This procedure is absolutely harmless for control animals, but triggers the fatal inbreeding phenotype in the L-line.

We were able to show that patterns in protein expression capture both constitutive line differences and specific changes associated with the lethal effect. (Figure 10). Presumably, the latter pattern is the signature of changes in protein expression levels in response to the expression of the lethal effect. Functional analysis showed that differentially expressed proteins were mainly involved in hexose metabolic process. These processes fit a pattern of build-up of energy stores and/or the production of cryoprotectants (glucose). Another feature of our protein set was that

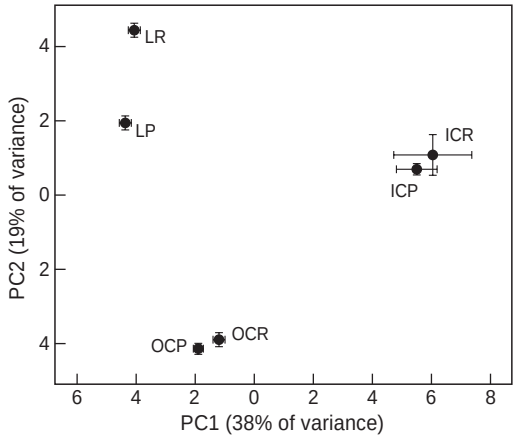

Figure 10. Results from Principle Component Analysis on protein expression levels. Shown are the positions (means and standard error of means) of all six combinations of line (Inbred Control IC, Conditional Lethal L and Outbred Control OC) by treatment (Permissive P and Restrictive R) on a PCA graph of the first and second principal component (PC1, explaining 38% of the variance and PC2, explaining 19% of the variance). There is a clear separation of all lines, and of treatment within the L-line.

many proteins were associated with lipid particles. Lipid particles are involved in lipid storage and lipid trafficking, and so may be involved in membrane remodelling or the build-up of energy stores. These features suggest that the inbreeding event is the result of a mis-expressed cold hardening response. In other words, it looks like the flies from the L-line kill themselves in their attempt to protect themselves from cold damage. This hypothesis needs yet to be validated, but if correct, this would be an intriguing example of the functional causes of inbreeding depression.

We are now in the process of analysing results from a transcriptomic analysis, and we have acquired the full genome sequence of all lines described in this study. Once completed, this will be the first detailed functional description of an inbreeding event.

Native insects and introduced plants – population differentiation, adaptation and host choice in insects occurring on native and non-native host plants

Kim Meijer, Menno Schilthuizen, Leo Beukeboom and Christian Smit[1]

1. Community and Conservation Ecology group, CEES, Groningen University

Nowadays many species have been transported and introduced into habitats outside their natural range, both intentionally as unintentionally. Many of those non-native species did not make it, they were unable to survive or reproduce. But part of them did and these provide a very good opportunity to study some important aspects of evolutionary biology. Since most non-native species are introduced without their natural preys, predators, parasites, etc. they provide new niches to colonize. This can give us inside into the processes of colonization and adaptation to new host and preys. We study these processes in plant _ phytophagous (herbivorous) insect systems in the Netherlands. The main questions we are working on are: to what extent do insects colonize non-native host plants; are insect populations diverging; are insects adapted to the species of host plant they occur on; and did they develop a preference to the host plant on which they occur? Several systems are studied to answer these questions.

Contrary to the general believe, many insect species can be found on non-native plants. During inventory we found close to 50 native phytophagous insect species foraging on non-native host plants, from different orders (Coleoptera, Diptera, Heteroptera, Hymenoptera and Lepidoptera) and different. As a follow up we studied differences in

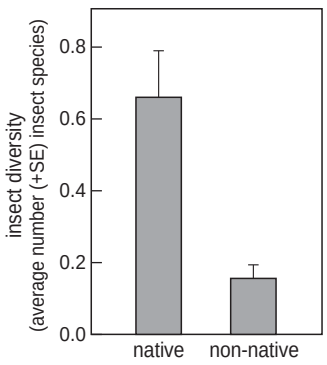

Figure 11. The overall insect diversity (number of insect species) on native and non-native host plants. Data collected in the Netherlands and Japan are combined.

Figure 12. A larva of *Rhagoletis alternata* (Diptera: Tephritidae) emerging from the berry of the non-native Japanese rose (*Rosa alternata*). These specialist fly larvae only occur in the berries of roses, both native and non-native.

species diversity and insect load (number of individuals) between native and non-native plants. An extensive literature study showed that in general both the species diversity and insect load were higher on native plants. However, the results differed between studies and methods used, and sample sizes were often (very) low. Next, a field study was set up and performed collecting data on species diversity, insect load (both number and dry weight of individuals) and herbivory damage in multiple native and non-native plant species. This study was simultaneously performed in the Netherlands and in Japan. Most plant species studied were either native in the Netherlands and non-native in Japan or vice versa. The results of this study were very clear, on native plants more species (Figure 11) and higher number of individuals occur and herbivory damage is higher.

At the moment we are working on the other research questions using several insect – plant systems. Population differentiation is being studied in leafminers flies (Diptera: Agromizidae), tepritid fruitflies (Diptera: Tephritidae, Figure 12) and leafbeetles (Coleoptera: Chrysomelidae); adaptation and host choice is being studied in the latter two.

Genetics of adaptation: Mating behaviour and reproductive strategies in *Nasonia* wasps

Maartje Giesbers, Bart Pannebakker, Louis van de Zande and Leo Beukeboom

Speciation, the process through which new species arise, is one of the main focuses of evolutionary biology. Speciation requires a population to be divided into two non-interbreeding populations. For this to be achieved an initial step of speciation is needed. In allopatric speciation this is achieved through the formation of a barrier between two sub-populations. In sympatric speciation, species diverge while inhabiting the same place, through processes like polyploidization or sexual selection. Over time, the two sub-populations are expected to diverge further through genetic drift or adaptation to their new environments through natural selection. Eventually they may become so differentiated from one another that they can be considered two different species.

However, species in nature are often incompletely isolated for millions of years after their formation. Therefore, mechanism must exist that help maintaining the species integrity to keep the incipient species separated. These are called isolating mechanisms, which are features of behaviour, morphology, or genetics that prevent breeding between species. Isolating mechanisms can be either post-zygotic (isolation occurs after fertilization has taken place) or pre-zygotic (isolation occurs before the zygote is formed).

Mating behaviour is an important pre-zygotic isolating factor. With a large variety of this behaviour in different species, it is a complex trait to study, and its genetic basis is still poorly known. We use the *Nasonia* genus of parasitoid wasps to study the genetic architecture of pre-zygotic isolation. This PhD study focuses on three *Nasonia* species that occur in micro-sympatry in Eastern North America: *N. vitripennis* is a cosmopolitan species that parasitizes several fly species. *N. giraulti* and *N. oneida* occur in Eastern North America and specialize on pupae of blowflies (Protocalliphora) commonly found in bird nests. *N. vitripennis* is reproductively isolated from both other species due to infection with species-specific strains of intracellular *Wolbachia* bacteria that cause cytoplasmic incompatibility between eggs and sperm. In the area of sympatry pre-zygotic isolation mechanisms might have evolved and/or strengthened in response to selection against hybrids. However, the importance of this process, called reinforcement, in the speciation process is still debated. Indeed, the species differ in male courtship behaviour but this may not be sufficient to prevent interspecific matings. *N. vitripennis* and *N. oneida* females show high mate discrimination against heterospecific males, but *N. giraulti* females do not. *N. giraulti* typically shows within-host mating whereas the other two species mate after emergence. Within host mating may have evolved to prevent interspecific hybridization, and is likely to be caused by an interaction between male courtship attempt and female acceptance.

Variation between lineages within species, differences between species, as well as the possibility of interspecies crosses, makes it possible to investigate the genetic architecture of the above mentioned behaviours. We hypothesize that these behavioural differences between the species are adaptive traits that have evolved to prevent costly interspecific matings leading to hybridization. We aim to identify the genes responsible for these traits with respect to their number, their

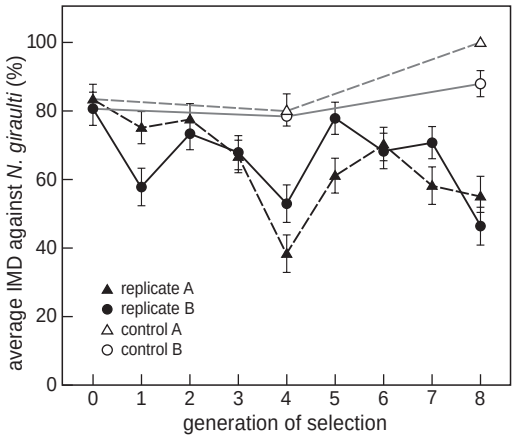

Figure 13. Interspecific mate discrimination of *N. vitripennis* females against *N. girault* males. For every consecutive generation in the artificial selection experiment the mate discrimination is plotted. Two replicate selection lines (A and B) are shown, including a control line for both replicates. Control lines were only observed every four generations, the selection scheme was similar to that of the selected lines except that random individuals were used to create the next generation.

effects and epistatic interactions. For this purpose, we will combine artificial selection, quantitative trait loci (QTL) analysis and gene expression profiling.

One of the main techniques used in this PhD project is artificial selection for interspecific mate discrimination. Through this process we want to establish lines that display a higher or lower interspecific mate discrimination when compared to non-selected lines from the same species. These selected lines can be observed for other (behavioural) traits as well, to see if there are correlated responses to the selection. For this purpose we used a typical *N. vitripennis* strain that shows a high mate discrimination against *N. girault* males. A kin selection regime – selecting the brothers and sisters of the observed females - was successfully conducted to decrease the mate discrimination against *N. girault* males, in only a few generations (Figure 13). In addition to this, we have observed the selected lines for mate discrimination against *N. longicornis* males and *N. vitripennis* (intraspecific) males. In these experiments the mate discrimination of selected females was also lower than the females from the control lines. The fast response to artificial selection could suggest that interspecific mate discrimination has a simple genetic basis, however more research is needed to make this conclusion. As lower mate discrimination in the selection lines against other species' males is observed as well, interspecific mate discrimination may not be species-specifically regulated, but instead may have a common genetic basis.

In addition to the selection experiment, recombinant males were created between *N. girault* and *N. oneida*. These species are genetically the most closely related *Nasonia*, but their behaviour is very different. With this species pair we hope to find the genetic basis of mate discrimination in the parts of the genome for which these two species differ.

The recombinant males were backcrossed with each pure species to create so-called clonal sibships. Female individuals from these sibships were observed for mate discrimina-

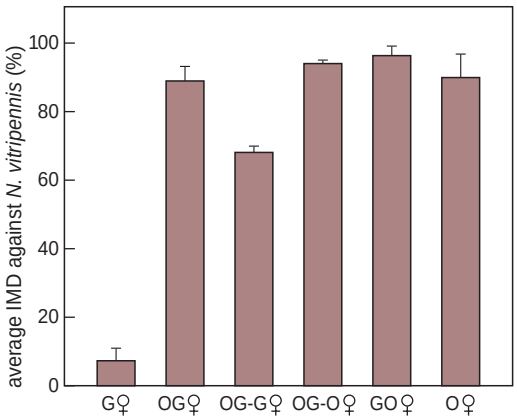

Figure 14. Interspecific mate discrimination against *N. vitripennis* males of pure *N. oneida* (O), pure *N. girault* (G), F1 hybrids (GO and OG) and recombinant backcrossed females (OG-O and OG-G).

tion against *N. vitripennis* males (Figure 14). From these experiments we can infer that mate discrimination is, at least partially, a dominant trait, because the hybrid and recombinant offspring behave more similar to *N. oneida* females – a species with high mate discrimination against *N. vitripennis*. Further analysis will be done to determine the heritability of the trait. The recombinant males created for this experiment will be genotyped and used in a quantitative trait loci (QTL) analysis. The genotype of the recombinant males and phenotype of the females from the sibships will be linked. Using a genetic linkage map on which all markers are positioned we aim to find the regions in the genome responsible for the mate discrimination traits.

Another reproductive trait studied in this PhD project is within host mating. For this trait, crossing experiments have been done to determine how the trait inherits in F1 hybrid individuals. The results show that within host mating is a complex trait that, in F1 hybrid females, is determined by both the maternal species and the paternal species. In addition, within host mating is influenced by the interaction between the female and the species of males present in the host. A QTL study will also be conducted to elucidate the genetic basis for this reproductive behaviour.

Latitudinal variation in photoperiodic induction of diapause in the parasitoid *Nasonia vitripennis* is genetically determined.

Silvia Paolucci, Louis van de Zande & Leo Beukeboom

Species that have a wide geographical distribution typically experience a broad range of variable environmental conditions and therefore must show large adaptive potential in order to be able to cope with such ecological diversity. In temperate zones, seasonality represents a major selective force driving local adaptation: individuals inhabiting different environments need optimal synchronization of their life cycle with seasonal cycles.

Diapause takes a leading part in the seasonal regulation of life cycles, it is a very important life-history trait (characterized by developmental arrest and lowered metabolic activity) present in many insect species (and other invertebrates) to survive adverse environmental conditions such as winter. As season length typically varies according to latitude; variation in several aspects of diapause is frequently observed and follows a latitudinal cline as a result of adaptation to local environments.

The induction and regulation of diapause are based on a combination of interacting genetic and environmental factors that affect endocrine pathways and physiological changes. Diapause response has been characterized in many insect species but the relative contribution of genetic and environmental effects is still under study. In particular, the genetic basis of variation in diapause response in different environments remains unknown.

This PhD project focuses on the variation in diapause induction in the parasitoid wasp *Nasonia vitripennis* and the genetics underlying this variation. *N. vitripennis* is a widespread species and diapause response in populations inhabiting different areas varies with the local seasonality. In *Nasonia*, the adult female is able to perceive environmental

Figure 15. Sampling locations in Europe, *Nasonia* field work 2009.

stimuli that signal upcoming adverse seasonal conditions and subsequently lays eggs that will develop into diapausing larvae. Development is resumed when favourable conditions are again available. Photoperiod represents the most reliable signal for upcoming seasonal change and is used by *N. vitripennis* as the most important cue for timing of diapause induction.

In order to investigate the genetic basis of this adaptive trait, we use natural populations of *N. vitripennis* collected in the field. In summer 2009 we collected populations in seven locations in Europe along a latitudinal gradient (Figure 15) and we characterized their photoperiodic diapause response in an experimental setup in which adult females were exposed to eight different photoperiodic conditions ranging from Light:Dark (L:D) cycles 8:16 to L:D 18:6 hours. For these females, lifetime diapausing offspring production was scored.

The results of this experiment show a latitudinal cline for several aspects of photoperiodic diapause response: (1) the effect of maternal age on diapause induction, (2) the overall diapause induction and (3) the critical photoperiod inducing diapause (Figure 16). In general, the incidence of diapause increases with latitude and Northern populations show a larger response to time of darkness compared to Southern populations. If the varia-

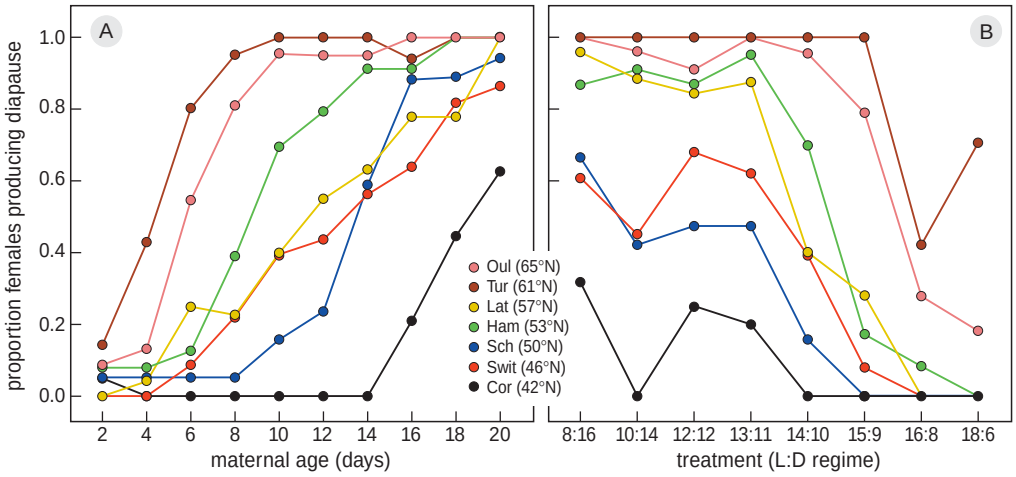

Figure 16. Latitudinal variation in diapause induction in *Nasonia vitripennis*. A: The effect of maternal age in L:D cycle 14:10. B: The effect of photoperiod in 10-day-old females.

tion observed has adaptive significance this diapause response may be the result of selection at different latitudes. This can be true only if diapause induction has a genetic basis.

To further investigate the genetic basis of diapause induction, we performed reciprocal crosses with individuals from two isofemale lines from the extremes of the cline (Oulu (65°N) and Corsica (42°N)). The photoperiodic diapause response of the F1 females was recorded and showed an intermediate value compared to the two parental lines (Figure 17). This result indicates that photoperiodic induction of diapause is genetically determined and is most likely a quantitative character with many genes of minor effect.

In order to localize these genomic regions, we established a set of microsatellite markers that are discriminative for the Oulu and Corsica lines. Using this set of genomic markers a QTL analysis is now in progress that takes advantage of the haplodiploid inheritance of the *Nasonia* genome. This will give information on the number and localization of loci involved in this adaptive trait and the underlying genetic architecture.

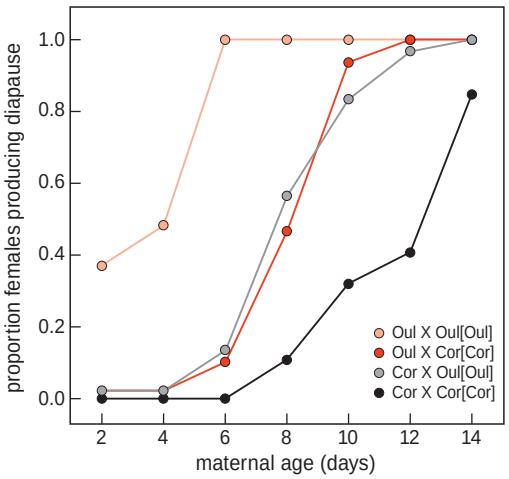

Figure 17. Diapause induction in *Nasonia vitripennis* (LD cycle 14:10) from North (Oul) and South (Cor) lines and F1 females resulting from reciprocal crosses between North (Oul) and South (Cor).

An additional approach that will be used is the candidate gene approach. In particular we will test whether clock genes, responsible for circadian activity (triggered by daily light: dark cycle), and that are known to have latitudinal allelic variation, are also involved in photoperiodic induction of diapause.

Publications 2010

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Marine Benthic Ecology and Evolution (MarBEE)

Group leader Prof. dr. J.L Olsen

Composition of the group in 2010:

Tenured Staff	**source**	**fte**	**period**
Prof. dr. J.L. Olsen	RUG	1.0	
Dr. B.D.H.K. Eriksson	RUG	1.0	
Prof. dr. W.T. Stam	RUG	0.3	080801-100201
Prof. dr. W.J.Wolff	RUG	Emeritus	-191231
Post-docs			
dr. M.K. de Boer	RUG	0.8	091001-130801
dr. J.A. Coyer	NWO-ALW	1.0	090901-120901
PhD Students			
Drs. S. Donadi	NWO-ZKO	1.0	090301-130301
Drs. M. Poortvliet	RUG	1.0	080401-120401
Drs. K. Sieben	RUG	1.0	070601-110601
Drs. S. Santos	NIOZ	1.0	070401-110331
Technical/Administrative Staff			
secretaries			
G. van Roon-ter Horst	RUG-CEES	0.5	until sep 2010
J. de Vries-Veldkamp	RUG-CEES	0.5	until sep 2010
H. Tjoelker	RUG-CEES	0.5	from sep 2010
technicians			
S.A. Boele-Bos	RUG	0.5	
J.H. Veldsink	RUG	1.0	
Adjunct Chairs			
Prof. dr. C.H.R. Heip	NIOO-CEME/NIOZ		

Overview of academic results

The theme of the Marine Benthic Ecology & Evolution group is "diversity in space and time", which is spread over two broad work areas, each with several projects: 1) population genetics-genomics, hybrid zones, speciation and phylogeography (Olsen lead); *and* 2) experimental community ecology, focusing on factors that promote resilience in complex benthic communities (Eriksson lead). There is also a sub-programme related to invasive species (Olsen and Wolff). The group utilizes field and laboratory methods with emphasis on links between ecological and evolutionary processes. There is a strong emphasis on the development and use of molecular tools in combination with manipulative field experiments.
(MarBEE: http://marbee.fmns.rug.nl)

Research Line 1 - From the MarBEE portfolio of population genetics-genomics, hybrid zones, speciation and phylogeography

Seagrass Ecosystems

Figure 1. The eelgrass *Zostera marina* in the Finnish Archipelago at a depth of 2 m. (Photo by C. Boström).

As a habitat-forming species, eelgrass (*Zostera marina*) (Fig. 1) belongs to a unique group of marine flowering plants that form the foundation of soft-sediment, coastal ecosystems. They are analogous to an underwater forest providing habitat for fish and invertebrates, as well as barriers to coastal erosion. Their continued world wide decline due to habitat fragmentation, fisheries pressures, pollution and climate change is of international concern. The ecological and societal importance of seagrass meadows, along with their experimental tractability, provides an unparalleled opportunity to test and integrate basic evolutionary and ecological theory. On the one hand, this includes understanding the effects of neutral (genetic drift) and deterministic (selection) processes on population connectivity and performance; and on the other hand, the ecological interactions governed by eelgrass diversity on community/ecosystem stability and resilience. Together they provide a framework for testing the influence of seascape-scale-specific environmental stressors that shape geographical patterns of connectivity and adaptive responses.

Senior research associate, **Jim Coyer** (ALW-open programme), continued his work on transcriptomic resilience of *Zostera marina* under climate change. Working with a team of colleagues and graduate students from the University of Münster and the IFM-Geomar in Kiel,

A 3-level factorial designed experiment was conducted in the Münster aquatron facility (Fig. 2). The experiment was designed to create an artificial heat wave, mimicking the Great European Heat Wave of 2003. Replicated genotypes from southern, naturally warm-adapted plants were compared with northern, naturally cold-adapted plants in replicated clines from both sides of the Atlantic. Plants were collected from two sites in the USA (Great Bay, NH; Waquoit Bay, MA) and two sites in Europe (Gabicce Mare, Adriatic Italy and Doverode, Denmark). The overall aim of the experiment was to compare

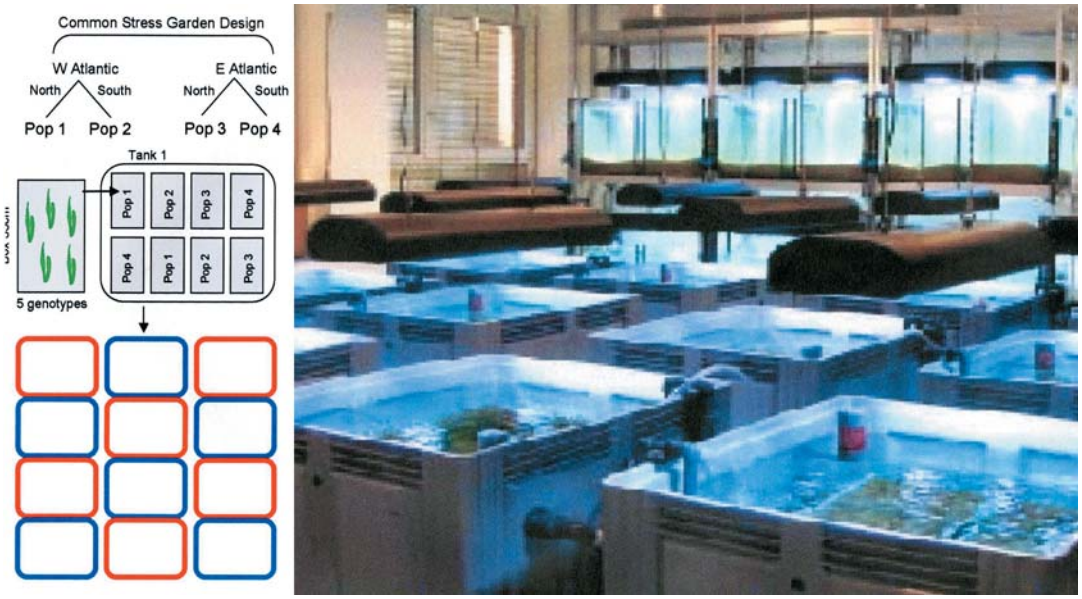

Figure 2. The Aquatron showing the experimental design used to simulate the heat wave of 2003.

Figure 3. *Zostera marina*. Mapman functional annotation of transcriptomic responses to an artificial heat wave created in the aquatron at Time 3 (upper panel) and recovery after Time 5 (lower panel) based on 1100 genes cross-referenced to rice proteins.

the transcriptomic responses and plant resilience to heat stress and recovery over time. Using the Illumina HiSeq platform, all 72 RNA libraries are currently being sequenced generating >10 Mb of sequencing/ library. Bioinformatics support is through collaboration with the Evolutionary Bioinformatics Group in Münster. Although these results are not yet available, some preliminary results based on a similar (but simpler) 2009 experiment show that both southern and northern populations respond to heat stress in a similar way, whereas the recoveries are substantially different with a delayed response of up to two weeks in the northern populations (Fig. 3).

In another set of experiments, PhD candidate **Steven Ferber** (Historical demography in *Zostera marina* throughout the North Atlantic, *Brede Strategie* 2003–2007) and PhD candidate **Katrina Oetjen** (shared PhD student with the IFM-GEOMAR in Kiel) used genome scans of EST-linked microsatellites to test for habitat specific selection between intertidal and subtidal ecotypes of *Zostera marina*. Two loci were identified, one linked to a nodulin gene which codes for proteins in the aquaporin group involved in water channels across cell membranes and related to osmoregulation; and the other linked to an acid phosphotase gene coding for proteins

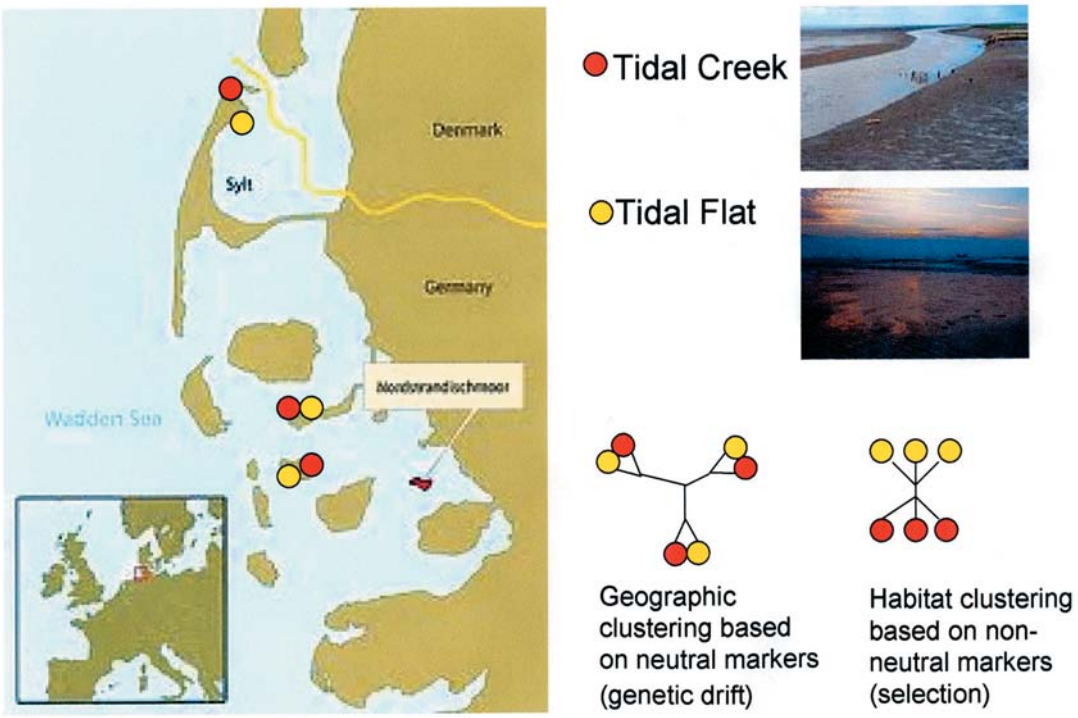

Figure 4. Evidence for habitat-specific selection in contrasting tidal creek (permanently submerged) and intertidal populations of *Zostera marina* populations revealed by genome scans from EST-associated microsatellite markers (Oetjen *et al.* 2010).

related to cell metabolism and signal transduction. Though still correlative, these markers group individuals by habitat rather than geographic position, as is the case when neutral markers are utilized (Fig 4).

Our work on seagrass systems also involves a second species, the dwarf eelgrass *Zostera noltii*, which is rapidly coming to dominate intertidal areas in more northerly latitudes where it was once relatively rare. PhD candi-

Figure 5. Characteristic feeding pits created by Brent geese (*Branta bernicla*) in a *Z. noltii* meadow off Sylt, Germany.

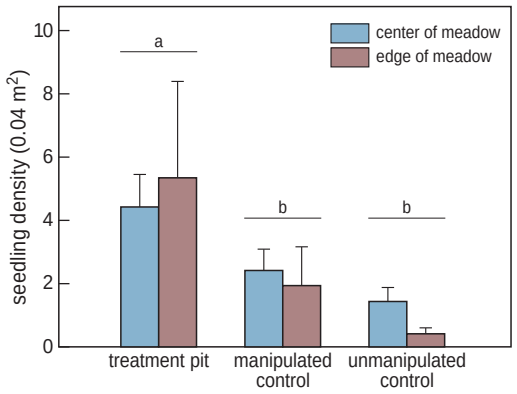

Figure 6. Seeding densities in treatment pits, manipulated controls and unmanipulated controls of dwarf eelgrass *Z. noltii* at center of meadow and at edge of meadow. Densities with differing letters (a or b) were significantly different (Turkey post hoc test, $p < 0.05$). Zipperle *et al*. 2010.

date **Andreas Zipperle** (*Population structure, demography and connectivity of Zostera noltii in the Wadden Sea, Ubbo Emmius 2003–2007*) published his fifth paper showing that 'treatment pits', mimicking natural feeding pits created by foraging geese (Fig. 5), 'captured' seeds and significantly enhanced seedling density relative to unmanipulated controls (Fig. 6). Establishment of artificial pits could be useful as a restoration strategy.

Group leader, **Jeanine Olsen** continues her coordination of the *Zostera marina* genome consortium. The species was accepted for genome sequencing (310 Mb) in the Community Sequencing Program of the Joint Genome Institute (JGI) in California in 2009. Obtaining the quality and quantity of genomic DNA required from a single genotype with minimal organellar contamination was challenging. Ultimately, a mega-clone was selected in the Finnish Archipelago near Korpoström (Figs. 7 and 8). Extensive EST libraries were completed in 2010 and the first genome draft is expected during the summer of 2011.

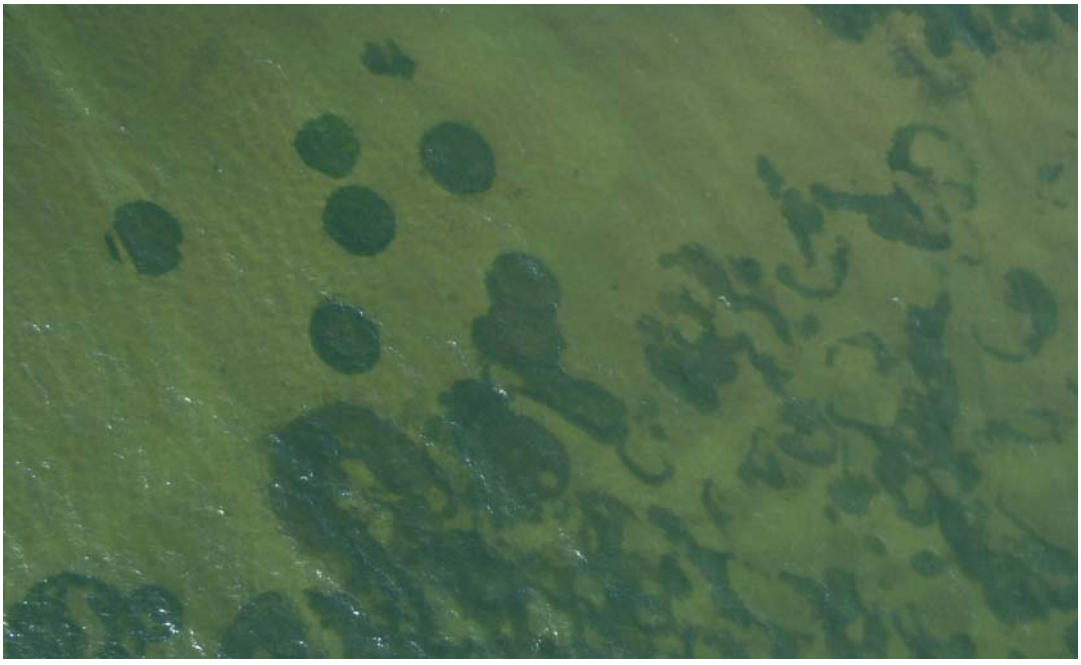

Figure 7. Aerial photograph of *Zostera marina* patches in The Finnish Archipelago using high contrast filters on a clear day. The "button-like patches" are large clones measuring 50+ meters in diameter (Photo by C. Boström).

Figure 8. Preparing to harvest one of the Zosmar clones shown in Fig. 7 (Photo by J. Olsen).

Jeanine is also actively involved in the development of genetic monitoring tools with a funded project to survey the California coastline including Baja, Mexico with colleagues from NOAA-Southern California. She is also participant and co-PI on a recently awarded ESF COST action: Seagrass Productivity: from Genes to Ecosystems (www.seagrassproductivity.com); and the Nordic Seagrass Network, whose goal is to develop a comprehensive biological assessment and economic valuation of seagrass ecosystem goods and services, leading to more functional legislation and, ultimately, an adaptive management program for the entire Baltic Sea and the Skagerrak-Atlantic coasts including The Netherlands, Germany, Denmark and Norway http://web.abo.fi/fak/mnf/biol/eco/nsn).

Phylogeography and climate change

The Trans-Arctic exchange, in which the Pacific marine biotia invaded the Atlantic, began ~3.5 BP and has been characterized by more than 30 glacial and interglacial periods. The Last Glacial Maximum (LGM) ended ~18,000 BP. The subsequent retraction of the glaciers raised sea levels and opened up new habitats for recolonization. Phylogeographic studies of many benthic metazonans and algae revealed key refugia (Maggs, Olsen *et al.* 2008 *Ecology*) and a number of testable hypotheses regarding expansion scenarios from east to west, and from south to north. Fucoid seaweeds have proven particularly informative as they are dominant habitat formers on all rocky Atlantic shores. An increasingly sophisticated understanding of how species have responded to climate change in the recent and more distant past also provides a basis for projecting responses to future climate changes.

For example, the LGM was not the main event that shaped geographic distribution for *Ascophyllum nodosum* (Fig. 9) but rather the penultimate Eems period ~300,000 BP. Most ecologists had assumed that *A. nodosum* became extinct on the North American side and was only subsequently recolonized from the European coast. However, estimates of effective population size (N_e) and migration rates based on both microsatellite and mitochrondrial markers, using coalescent methods, suggests similar N_e on both coasts, larger refugia on both sides and little recent contact between European and American populations despite the potential for long distance dispersal (Olsen *et al.* 2010). Populations of *A. nodosum* in Brittany represent the center of the range and the main genetic hotspots of diversity. However, these populations are analogous to "old growth forests", which means that they experience little recruitment and are more than ordinarily susceptible to impacts (such as harvesting) and other threats to biodiversity.

The genus *Fucus* (Fig. 10) presents possibly the most interesting case for studying phylogeography, adaptation and speciation in the rocky intertidal because its many species exhibit both allopatric and sympatric distributions in the Atlantic; and distinct zonational gradients across the intertidal. Given the effects of the LGM, as a recent physical "shaper" of the distribution of genetic diversity, we have been most curious about its species diversity and rapid adaptation over the past few thousand years (and perhaps even more recently).

In 2010 we completed comprehensive phylogeographies (>4000 individuals from >100 locations covering the total range of the taxon) for the four main species lineages: *Fucus distichus* (Pac-Atlantic, cold-temperate-Arctic; low intertidal), *F. vesiculosus* (warm

Figure 9. *Ascophyllum nodosum* survived the LGM on both sides of the Atlantic with similar effective population sizes (Photo by J. Olsen).

Figure 10. Mixed bed of *Fucus* species (Photo by J. Coyer).

and cold temperate; mid-intertidal; subtidal Baltic) and *F. spiralis* (warm temperate; high intertidal). *F. serratus* (cold-temperate; low intertidal, subtidal was completed by Hoarau *et al.* 2008 *MolEcol*).

Fucus distichus is the only species that occurs in both the North Pacific and North Atlantic. The work is significant as global climate change has lead to significantly reduced ice cover in the Arctic with the eventual year-round connection of the North Pacific and North Atlantic increasing the possibility of genetic interactions among and within species. Our work showed that North Pacific *F. distichus* colonized the North Atlantic on at least two different occasions from two different regions between the opening of the Bering Strait and onset of the LGM (Fig. 11). Japanese populations probably arose from a single recolonization event from SW Alaska. Within the North Atlantic, the LGM also forced the species into at least two glacial refugia: the Nova Scotia/Newfoundland region and northern Norway. The presence of two high frequency haplotypes in the central Atlantic suggests the possibility of colonization from other refugia that are now too warm to support *F. distichus*.

Fucus vesiculosus is the most widespread species and occurs sympatrically with it's sister species, *F. spiralis* in the mid-latitudinal range. *F. spiralis* extends further south to Morocco. Nothing was known about the phylogeography of the Fv/Fsp lineage, particularly in the southern region of the distribution, where uninterrupted survival, hybridization and expansion would have been common. The study turned out to be unexpectedly complex,

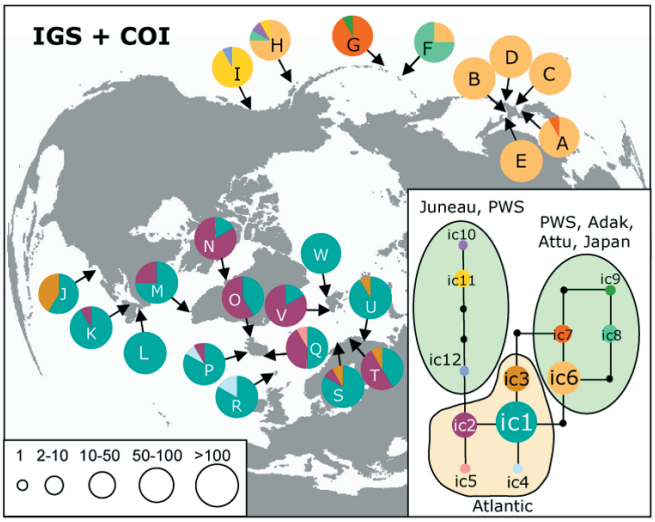

Figure 11. Distribution of combined mtDNA intergenic spacer (IGS) and COI haplotypes and the haplotypes network for *Fucus distichus*. Manuscript submitted.

presenting challenges to sampling, identification and data analysis. To begin with, three genetic entities of *F. spiralis* were recovered (Fig. 12). In northern and sympatric populations, the presence of "*F. spiralis* Low" in the mid-intertidal and "*F. spiralis* High" in the high-intertidal was confirmed and both co-occurred with the sister species *F. vesiculosus*. The third and newly-discovered entity, "*F. spiralis* South", was present mainly in the southern range, where it did not co-occur with *F. vesiculosus*. The South entity diverged early in allopatry, then hybridized with *F. vesiculosus* in sympatry to produce *F. spiralis* Low. Ongoing parallel evolution of *F. spiralis* Low and *F. spiralis* High is most likely due to habitat preference/local selection and maintained by preferentially selfing reproductive strategies.

Contemporary populations of *F. spiralis* throughout the North Atlantic stem from a glacial refugium around Brittany involving *F. spiralis* High; *F. spiralis* South was probably unaffected by glacial episodes. Exponential population expansion for *F. vesiculosus* began during the Cromer and/Holstein interglacial

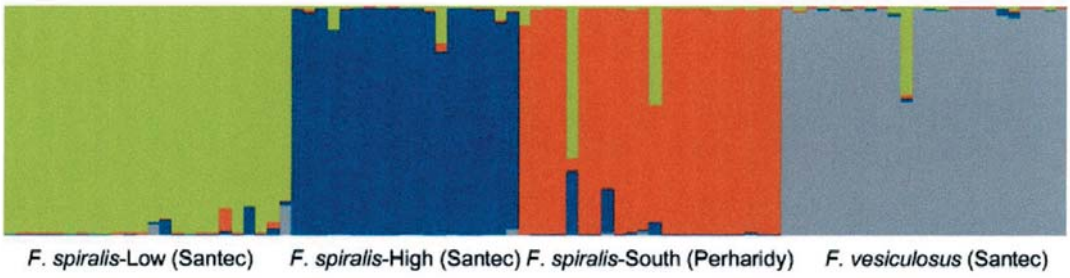

Figure 12. Verification of four *Fucus* entities based on a Bayesian analysis with STRUCTURE v. 2.2 (Pritchard *et al.*, 2000) and 12 microsatellite loci. Each vertical bar is the multi-locus genotype of one individual and colors represent the proportion of the genotypes assigned to each genetic group. Green, Fsp-Low (Santec); Blue, Fsp-High (Santec); Orange, Fsp-South (Perharidy); Grey, *F. vesiculosus* (Santec); parameters: K=4; MCMC=1,000,000. Manuscript submitted.

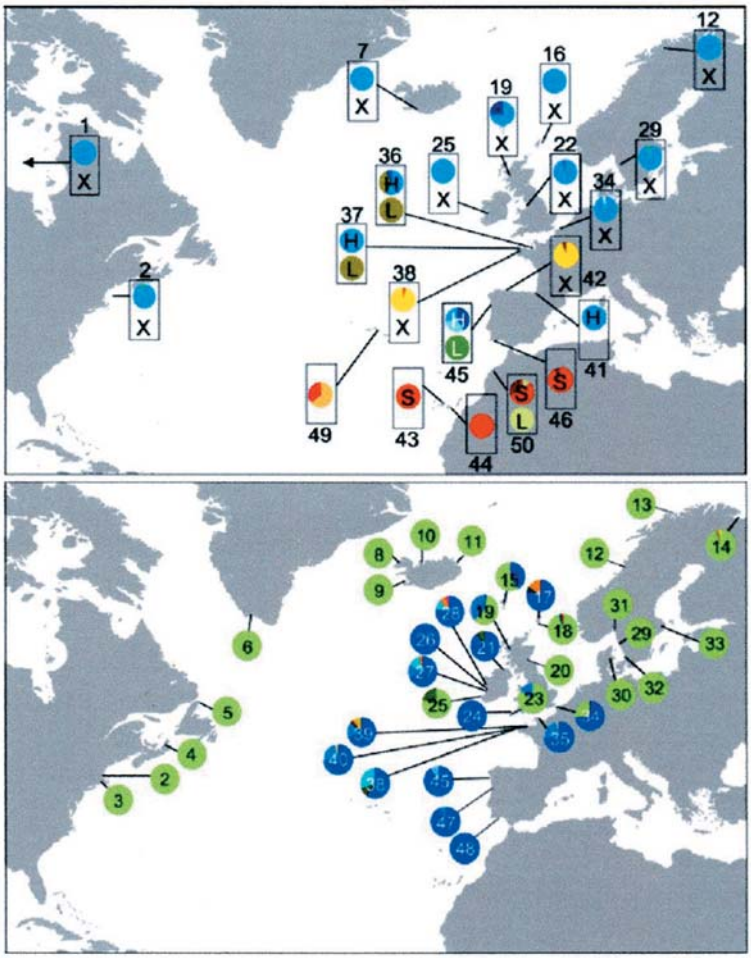

Figure 13. Distribution of *F. vesiculosus* and *F. spiralis*. Top. *F. spiralis* mtDNA haplotypes based on identification using five nuclear microsatellite loci. Each box contains two circles representing genetic identifications as low (L) and high (H) shore – this microsatellite-based identification was only conducted on sites whose circles contain a letter, identifying the circle as high (H), low (L) or south (S) genetic types; boxes with one circle and an "X" represent a location where only the high shore was sampled and verified. Bottom. *F. vesiculosus* and *F. spiralis* mtDNA haplotypes based on identification using 12 microsatellite loci. For both species, size of the circles is NOT proportional to the number of individuals with the given haplotypes. Location numbers are presented in the paper. Green represents *F. vesiculosus* with no variation in the north and west; blue represents the spiralis grouping including hybrid and introgressed entities. Manuscript submitted.

period (300,000 to 200,000 y BP). Following the LGM, a single mtDNA haplotype from a glacial refugium in SW Ireland colonized Scandinavia, the Central Atlantic islands, and the W Atlantic (Fig 13). The dynamic radiation of *Fucus* stems in part from its predominance in numerous micro-environmental gradients over spatial and temporal scales, which promotes divergence (as has been shown for the subtidal *F. radicans* in the Baltic in a timeframe of <4000 years (Pereyra 2009 *BMC EvolBiol*). Microspeciation in the *Fucus vesiculosus-spiralis* complex is similar and ongoing, as shown in Fig. 14.

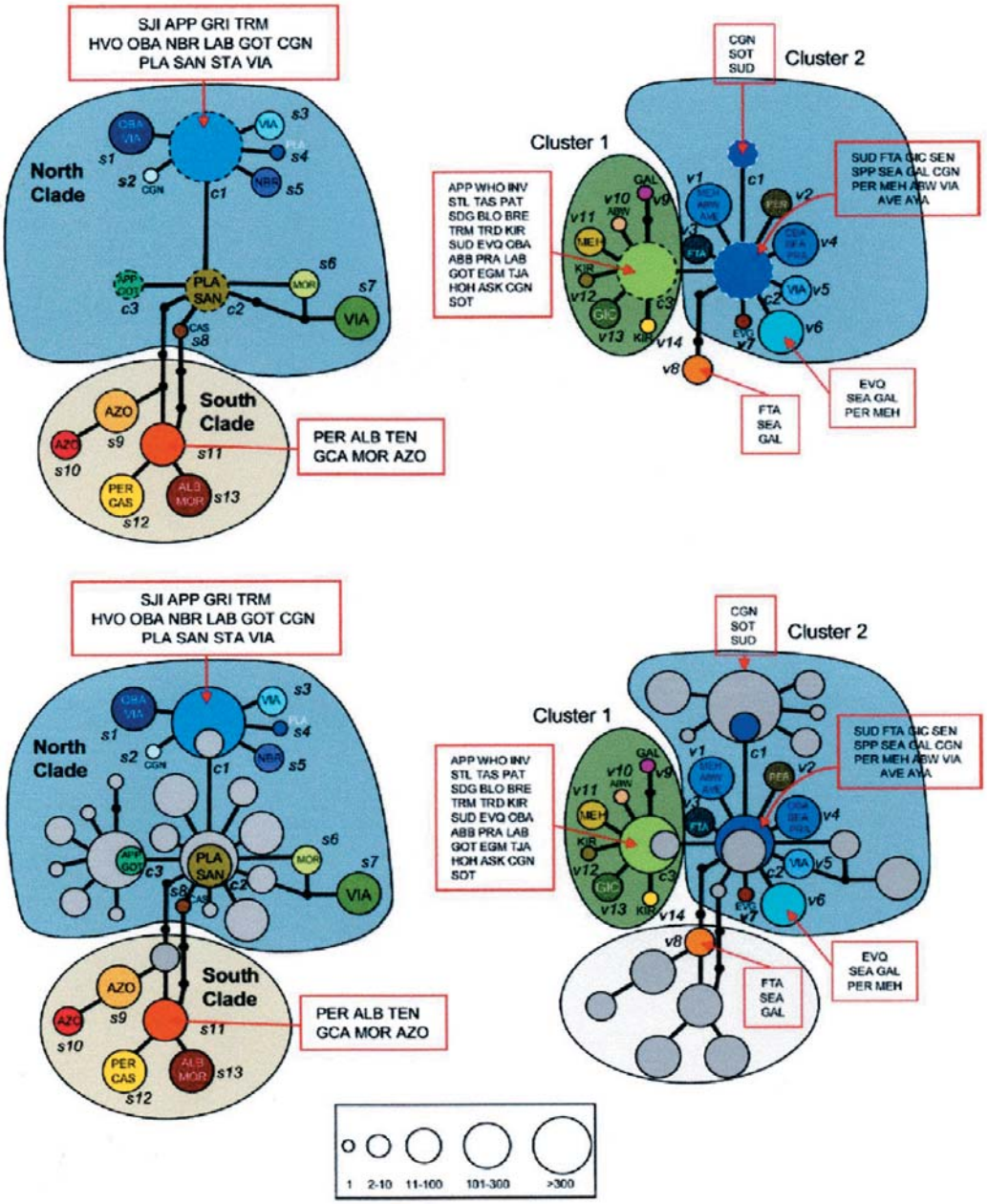

Figure 14. Statistical parsimony network of *F. spiralis* and *F. vesiculosus* based on mtDNA IGS haplotypes. Each circle represents one haplotype and size is proportional to the number of individuals with the given haplotype as shown in legend. Each branch between two nodes represents one mutational step and small black nodes represent haplotypes with intermediate mutation steps not found. Abbreviations for place names and haplotypes are not shown here. Colors can be compared within a species, but cannot be compared among species. Three haplotypes (c1, c2, c3) are shared among *F. spiralis* and *F. vesiculosus* and are indicated with dotted lines in the upper panels and overlapped in the lower panels. Upper left: *F. spiralis* haplotypes are shown in color (s1-si13), lower left: *F. spiralis* haplotypes overlaid on *F. vesiculosus* haplotypes (grey, unlabeled). Upper right: *F. vesiculosus* haplotypes are shown in color (v1-v14), lower right: *F. vesiculosus* haplotypes overlaid on *F. spiralis* haplotypes (grey, unlabeled).

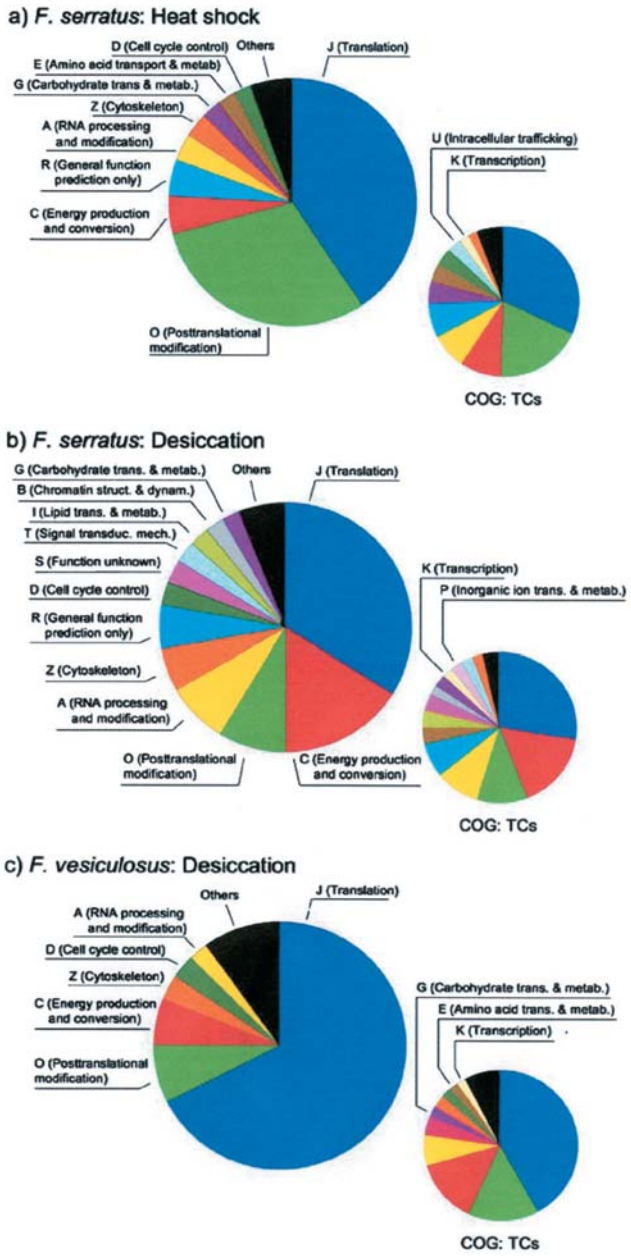

Figure 15. Pie charts showing major functional categories (COGs) for the ESTs (large circles) and tentative consensus (TC) sequences (small circles) from a. *F. serratus* heat shock/recovery, b. *F. serratus* desiccation/rehydration, and c. *F. vesiculosus* desiccation/rehydration libraries.

Rapid adaptation of *Fucus* species and entities to different micro-stressors is also being studied using parallel replicate habitats across the intertidal gradient. We have initiated this work (Pearson, Olsen 2010 *MarBiotech*) as part of our adaptive population divergence line where we have developed EST libraries for *F. vesiculosus* and *F. serratus* (Fig15.). In the coming year we will compare responses of *Fucus* species across their zonational gradient looking for evidence of rapid selection.

The world of mobulid rays

PhD student **Marloes Poortvliet** (*Population connectivity and ecology of mobulid rays, Top-Master-PhD scholarship 2007–2011*) worked extensively in 2010 to obtain specimens from throughout the world (Fig 16). Because there is no specific ray-fishery, they are either reported as bycatch or not at all. Those bycatch estimates that exist suggest that tens to hundreds of thousands may be lost annually, especially in combination with shark fisheries. Obtaining samples has proven challenging to say the least. Results from a survey of 10 locations and ~400 individuals using microsatellites and two mitochondrial loci reveal a strong dominance of one global haplotype and many regionally-specific haplotypes. The degree to which these finding suggest a recent expansion of *Mobil japanica* following a bottleneck of unknown cause in the past vs. female-sex-biased dispersal over very long distances remains unclear as sexing samples is difficult and generally not available. Tagging studies along the Baja California coast and further south suggest that these pelagic feeders follow the food. However, it remains unclear as to weather long-distance migration (possibly traversing the tropical Pacific) are characteristic of this group.

Figure 16. Rays are threatened worldwide because of their vulnerability to bycatch in open ocean fisheries yet little is known about their natural history. *Mobula japanica* is one of the most threatened (Photo by M. Poortvliet).

Invasive species

As a continued threat to biodiversity, documenting the extent and provenance of invasive species including propagule pressure continues to be important in helping to understand population dynamics of source and invading populations. This last year, our work shifted away from algae to tunicates (Haydar et al., 2010) and oysters (Troost 2010).

Newly minted PhD, **Deniz Haydar** (*NWO-ALW, SUSUSE, 2003–2007*) comprehensively reviewed invasion biology in the North Atlantic, brinfing together extesively historical and contemporary records in her thesis, What is natural? The scale and consequences of marine bioinvasions in the North Atlantic Ocean. As a side project, she used the barcoding mitochondrial gene COI to survey the widespread tunicate (sea-squirt), *Molgula manhattensis*, which has a disjunct amphi-Atlantic distribution and a recent history of world-wide introductions. Although native to North America, it remains unclear as to whether it was introduced or is native to Europe (Haydar *et al.* 2010) (Fig. 17). Although additional sampling and markers might reveal the presence of putative private European haplotypes in America, the low

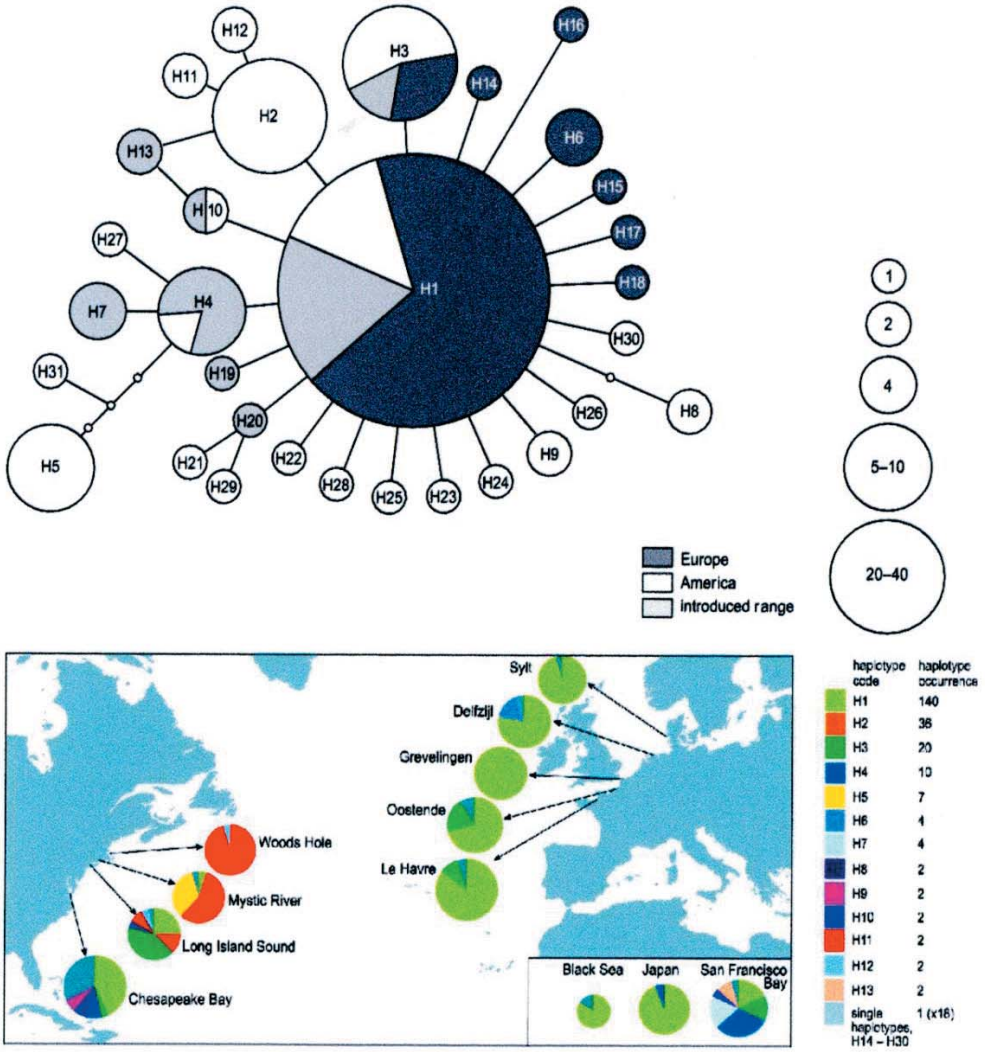

Figure 17. Haplotype network for *Molgula manhattensis*. Haplotype circles are proportional to haplotype frequency. European haplotypes (or proportions of haplotypes occurrence) are indicated in black, NE American haplotypes are white and haplotypes in the introduced range are gray. The lower panel shows the distribution of CO1 haplotypes per sampling location.Gray-shaded areas are the cumulative proportion of single, unique haplotypes per location.

European diversity may also be explained by low effective population size and a recent expansion; or by low propagule pressure of anthropogenic introduction. Absence of medium-to-high frequency American haplotypes in Europe may be the result of exclusive anthropogenic transport from southern American ports, or long-term residence. This case illustrates the difficulty (in many instances) of using a single marker yet this is commonly what is done in such surveys. Once again, it is important to remind ourselves that if a marker shows a definitive pattern, that is fine; but if it does not, then absence of evidence is not evidence of absence.

Research Line 2 –From the MarBEE portfolio on experimental community ecology

The overall aim of this research line within MarBEE – led by tenure tracker **Britas Klemens Eriksson** – is to understand how biodiversity loss (in the broad sense) affects the resilience of communities. Focusing on experiments in natural conditions, the group tests how trophic levels, functional groups and the number of species determine the capacity of marine benthic communities to tolerate disturbances or stress.

Biodiversity and resilience in the Wadden Sea system

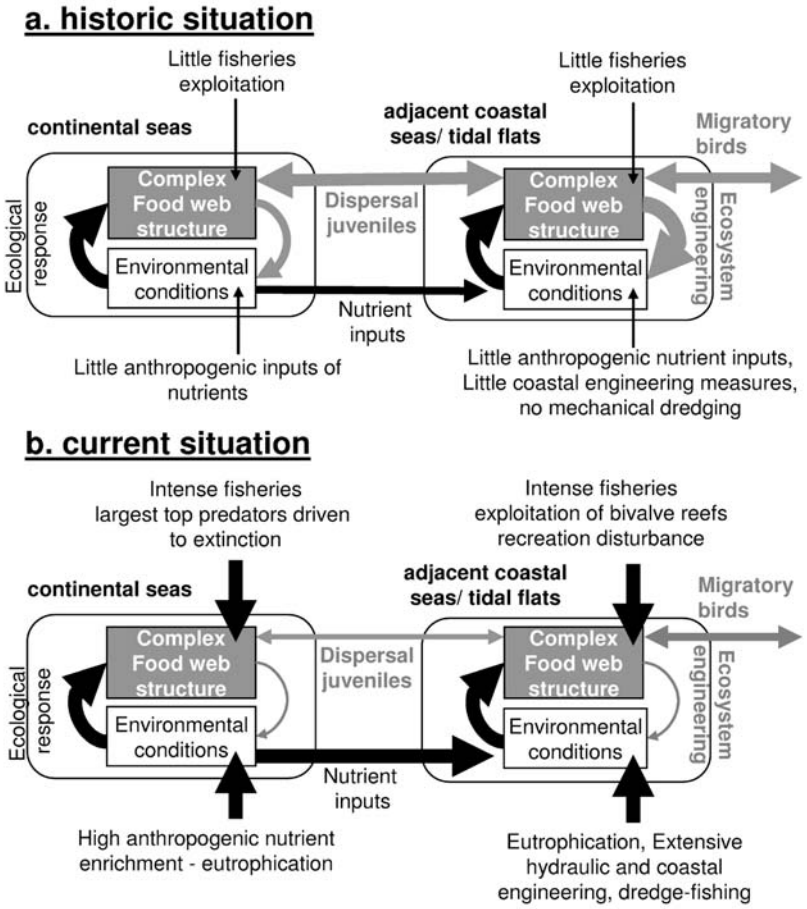

Figure 18. Changes in the relationship between species and environmental factors of a coastal system, here the Wadden Sea, within a meta-ecosystem (North Sea and coastal wetland) context. a) historic conditions: strong internal biotic feedback control of environmental conditions. b) current situation: loss of internal biotic feedback, replacement by external forcing of human interventions in adjacent river and continental off-shore ecosystems.

Figure 19. Experimentally constructed cockle banks on Schiermonnikoog. Each plot is 5 x 5 m and includes 25000 added cockles (Photo by S. Donadi).

In 2010 group leader **Britas Klemens Eriksson** published a profile paper presenting the idea that a 'human induced regime shift' has changed the Wadden Sea from an internally to an externally regulated ecosystem (Eriksson *et al.* 2010). He and colleagues proposed that in historic times, ecosystem dynamics and community distributions in the Wadden Sea were dominated by processes related to sediment stabilization through the presence of seagrass and mussel beds. Over the past 100 years, however, loss of seagrass (*Zostera marina*) combined with the sequential exploitation of oysters (*Ostrea edulis*), then mussels (*Mytilus edulis*) and then cockles (*Cerastoderma edule*) has led to large-scale sediment disturbance resulting in the dominance of sediment-destabilizing polychaete worms instead. The result it that today, the Wadden Sea is dominated by external drivers in the form of human induced changes in nutrient dynamics, constrained hydrodynamics as a result of human engineering projects (e.g., the construction of the afsluitdijk in the 1930s), climate change and sediment disturbance due to bottom trawling (Fig. 18). This general idea forms the basis of a collaborative project within CEES coordinated by Eriksson, funded by the NWO program for ocean and coastal research (ZKO).

PhD student **Serena Donadi** (*Consequences of ecosystem engineers for the trophic structure and recovery potential of the Wadden Sea ecosystem, ALW-ZKO*) is studying interactions between benthic soft-bottom communities dominated by ecosystem engineers and local resource conditions in the Wadden Sea. We specifically study engineering effects of sediment destabilizing ecosystem engineers (poly-

Figure 20. Lugworm addition on Schiermonnikoog by two of many volunteers during the experimental set up in 2010 (Photo by B.K. Eriksson).

chaete worms) and sediment stabilizing suspension feeders (mussels, cockles), and how such relations may be modified by human impacts on sediment stability (dredging, trawling, engineering). In 2010 Serena started a large scale experiment on a tidal flat on the Wadden island of Schiermonnikoog, where she constructed communities dominated by different ecosystem engineers. Two million cockles (Fig. 19) and 200000 lugworms (*Arenicola marina*) were added to different plots (Fig. 20). She also worked with local fishermen to test effects of sediment disturbance by traditional hand cockling (Fig. 21). This unique large scale experiment covers 2400 m^2 of the intertidal and will run for at least three years.

Figure 21. PhD student Serena Donadi demonstrates traditional cockle fishing (Photo by I. van Gerwen).

Biodiversity and resilience in the Baltic system

Research by **Britas Klemens Eriksson** and colleagues on effects of declines in larger predatory fish have previously demonstrated that an 80% decline in perch and pike stocks (*Perca fluviatilis* and *Esox lucius*) has induced a mesopredator release of a medium sized coastal fish, the three-spined stickleback (*Gasterosteus aculeatus* L.); and that together with nutrient enrichment, increases the production of filamentous algae in the Baltic Sea. In 2010 they published results indicating that the described declines in coastal predatory fish may have been triggered by a large-scale reproductive disturbance in the outer zones of the archipelagos on the Swedish east coast (Ljunggren *et al.* 2010). The reproductive failure appears to have been caused by limited food availability for larvae, since recruitment success correlates strongly with the abundance of important zooplankton prey groups

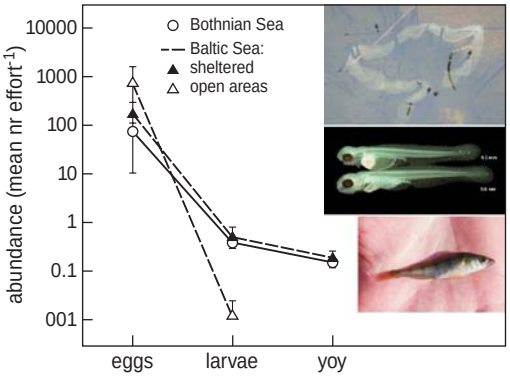

Figure 22. Abundance of different life-stages of perch during the summer of 2003 in the Bothnian Sea, and sheltered and open areas in the Baltic Sea proper.

and the recruitment failure target size classes of larvae that are in the process of switching from feeding off their yolk sac to active feeding in the water column (Fig. 22). This has major management implications. A proposal

Figure 23. Setting up a large-scale diversity and exclusion experiment in the Baltic (Photo by K. Sieben).

Figure 24. Close up of treatment cages (Photo by K. Sieben).

to incorporate the results of our work into governmental management plans for the Baltic Sea are now being evaluated by the Swedish government.

PhD student **Katrin Sieben** (*Consequences of predator declines for coastal resilience to eutrophication RUG-beurs*) have focused on exploring the effects of the described changes in trophic structure in the Baltic Sea on ecosystem function by using combinations of experiments that manipulate the consumer food-web (Fig. 23). Eutrophication and fishing are among the most important drivers of biodiversity loss and attendant marine processes at a global scale and she has therefore specifically looked at consequences of functional

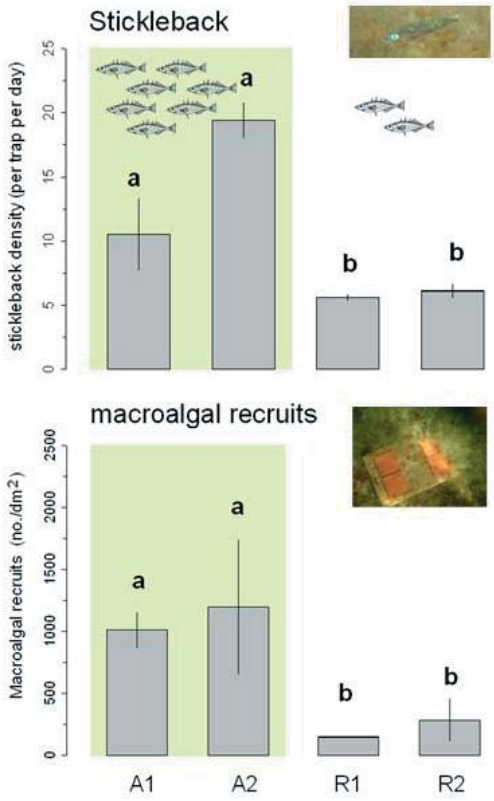

Figure 25. Stickleback (and macroalgae recruit densities (number/dm^2) in stickleback addition (A1 and A2) and removal enclosures (R1 and R2). Letters a and b denote significant differences $p < 0.05$ (means ± SE, N = 2). The grey area indicate enclosures where stickleback where removed (R1 & R2).

diversity in predator and prey communities (Fig. 24) and nutrient enrichment for the intensity of trophic cascades. During 2010, she finalized work demonstrating that coastal trophic cascades induced by loss of predatory fish are dependent on nutrient enrichment (Fig.25) (Sieben *et al.* in press). Through this experimental work we have been able to provide definitive evidence that effects of overfishing and eutrophication reinforce each other and thereby create "double trouble" for managers of coastal ecosystems.

Biodiversity and resilience in the laboratory

Post-doc **Karin de Boer** (*The relationship between biodiversity and resilience in source-sink meta communities NWO-ALW*). A major aim of her research line is to predict consequences of species loss for the ability of communities to cope with environmental stress and disturbances. Together with Leibniz-Institute for Marine Sciences (IFM-Geomar) in Kiel (Matthiessen) we have established meta-communities consisting of connected habitats of marine benthic microalgae in the laboratory. We expose these natural communities to

Figure 26. Experiment testing the resilience of meta-communities of benthic microalgae to temperature disturbances depending on community composition. A) one meta community: the different flasks are local communities with different light conditions and are connected by dispersal. B) the experimental set up per temperature treatment: meta communities with green labelled flasks were run at control temperatures of 16 degrees and red labelled flasks were exposed to a temperature disturbance in the form of a heat-wave.

temperature disturbances by mimicking real heat wave conditions that have been measured in the field. This allows us to test for patch-dynamics by including environmental disturbance and dispersal. Disturbance and dispersal are natural processes that promote species coexistence – but have been neglected processes when it comes to biodiversity-ecosystem function experiments. In 2010 Karin ran a first large experiment testing how species evenness influences responses of a meta-community to temperature disturbance. The experiment consisted of 96 meta-communities sampled at different time intervals, generating a total of 576 samples of microalgae and bacteria (Fig. 26). Preliminary results indicate that communities that are dominated by one highly productive species (low evenness) have very low resilience to temperature disturbances. We detected no recovery of these communities over five asexual generations. In contrast, communities with more even species distributions showed a strong recovery potential. Thus, our results suggest that the relative abundances of species is an important property for communities to cope with climate change.

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Creuwels, J.S.C. 2010. Breeding ecology of Antarctic Petrels and Southern Fulmars in coastal Antarctica. PhD thesis, Unversity of Groningen, The Netherlands. (Promotor: Prof. dr. W.J. Wolff).

Haydar, D. 2010. What is natural? The scale and consequences of marine bioinvasions in the North Atlantic Ocean. PhD thesis, University of Groningen, The Netherlands. (Promotor: Prof. dr. W.J. Wolff).

Microbial Ecology

Group leader Prof. dr. J.D. van Elsas

Composition of the group in 2010:

Tenured Staff	**source**	**fte**	**period**
Prof. dr. J.D. van Elsas	RUG	1.0	
Dr. J. Falcao Salles	RUG	1.0	
Post-docs			
Dr. A.M. Kielak	RUG	0.9	100415-110414
Dr. R.M. Nissinen	Marie Curie grant	1.0	090401-110401
Dr. A.V. Semenov	NWO	1.0	080915-110915
Dr. P. Stevens	RUG	0.9	090501-111107
PhD Students			
Drs. M.C. de Cassia Pereira e Silva	NWO	1.0	080915-120915
Drs. M.S. Cretoiu	RUG	1.0	090915-130914
Drs. P.R Hardoim	UE bursary	1.0	060901-100901
Drs. E.F. Hoogwout	NWO	1.0	070715-110715
Drs. O. Inceoglu	NWO	1.0	070907-110907
Drs. R. Nazir	NUFFIC bursary	1.0	070901-120918
Drs. C.A. Mallon	bursary	1.0	090901-130831
Drs. U. Nunes da Rocha	bursary	1.0	060315-100315
Drs. P.E. Puentes Tellez	NWO	1.0	090901-110228
Ing. P. Stevens	RUG	0.9	090515-110515
Drs. M. Zhang	bursary	1.0	101001-140930
Guest workers			
Dr. L. Procopio da Silva	Brazil	1.0	090301-100228
mw. N. Sarigul, MSc	Turkije	1.0	100701-101231
Technical/Administrative Staff			
secretaries			
mw. M. Dusseljee	RUG	0.6	
mw. J. de Vries-Veldkamp	RUG	0.5	
mw. B. Zand Scholten	RUG	0.8	
technicians			
Ing. J.K. Brons	RUG	1.0	

Introduction

The research programme in the Microbial Ecology Department focuses on the adaptation of prokaryotic microorganisms to a variety of environmental niches, and the ways in which the genomes inolved allow plasticity to enhance fitness. The heterogeneous and fluctuating conditions in most current ecosystems, microbial competition and adaptation to biotic and abiotic factors, and consequent evolution, have resulted in the fascinating microbial diversity that is currently found in natural habitats. Microorganisms inhabit almost every thinkable ecological niche on the Planet, and the diversity in the microbial world is daunting by its sheer magnitude Only a minor fraction of this diversity has been accessed to date.

Understanding the ecology of the diverse microbial communities and their interaction with each other and the environment thus represents a major challenge to current microbial ecology research. At present, most of the studies of microbial diversity and function focus on organisms at the community level, using, for instance, the 16S ribosomal RNA (rRNA) gene sequence as a marker of microbial diversity. Thus, an inventory is made of the microbial diversity extant in target ecosystems (soils, aquatic systems), and the putative ecophysiological role of members of that community is inferred from the data. However, finding similar populations of species in similar or different environments does not necessarily mean that these are also functionally identical. This is caused by the fact that microbial populations are genetically much more diverse than can be concluded from the diversity of 16S rRNA genes, which belong to the core set of bacterial genes. In fact, genetic diversification within microbial populations is the key step in microbial adaptation and evolution. Several mutational processes, ranging from horizontal gene transfer through genomic rearrangements to small mutations, may yield variants with traits that enhance fitness. The genetic diversity within a community or population of cells may thus often be missed by 16S rRNA gene-based culture-independent methods, although we do not fully understand the limits to using 16S rRNA genes to infer community function. This lack of sound information provides the rationale for current innovative work that aims to address key questions such as “how well does community structural diversity reflect functional diversity” and “how does horizontal gene transfer interfere with the putative relationship between structural and functional diversity”.

Hence, questions in respect of how bacteria adapt genomically to the environmental challenges they encounter, on what occasions they show adaptive responses, how horizontal gene transfer impacts on the adaptational process and at which speed adaptations occur, represent main topics addressed in the Microbial Ecology research programme. In terms of ecosystems, the focus is on soil and soil-related habitats, as (1) in the light of the multitude of niches and gradients present in this habitat, there are challenging and largely unstudied processes of niche adaptation and niche differentiation to be found in this habitat, and (2) a number of key and major drivers of microbial activity, including adaptational activity, such as plants and soil fungi, are available. Several other projects deal with non-soil systems, such as freshwater and surface marine waters (sponges).

Overview of academic results

Adaptation of *Escherichia coli* to heterogeneous conditions

This project aims to establish the direction and level of divergence of *Escherichia coli* under conditions of fermentation in simulated industrial systems. The emphasis is on unraveling of the genetic drift and physiological changes that the organism undergoes, which allow it to be superior (fitter), occupy a different niche and lead to a robust fermentation process. Thus, this project is simulating the conditions of a fermentor as well as using a small-scale fermentor system to assess heterogeneity. Long-term continuous cultivations are performed with different selective operational forces and samples are taken at regular intervals for monitoring of the genetic drift, focusing on the (bottleneck) conditions that determine bacterial growth and heterogeneity.

In 2010 P. E. Puentes Tellez has developed a long-term experimental evolution experiment obtaining 1000 generations of *E. coli* MC1000 growing under different oxygen regimes. The physiological characteristics of the emerged forms have been analyzed. The fitter forms have been identified and selected by growth competitions against the ancestral strain and will be used for further genetic analysis. The next phase of this experiment includes genetic characterization with several molecular and microbiological approaches (e.g. genetic fingerprinting techniques, microarrays) in order to identify genomic rearrangements, mutations and physiological diversity of the subpopulations obtained.

By the end of this study, we will know to what extent genetic heterogeneity occurs in spatially structured fermentor systems. This study will bring new insights in the underlying mechanisms driving heterogeneity of *Escherichia coli* in spatially structured fermentation systems. The knowledge will be useful for establishing a platform for more robust fermentation process and for controlling heterogeneity in industrial processes.

Adaptational processes that allow *Ralstonia solanacearum* to survive under temperate climate conditions

The Gram-negative bacterium *Ralstonia solanacearum* is a plant pathogen that causes bacterial wilt of a variety of plants. It is responsible for great economic losses in agriculture. The bacterium has increasingly been observed in temperate climate zones. The aim of this study was to assess whether genetic changes had occurred in local strains and whether such changes can be related to enhanced fitness under local conditions. *R. solanacearum* biovar 2, the causative agent of potato brown rot, probably originates from the cradle of potato, the Andes and from there has incidentally spread to different regions of the world. In the Netherlands, bacterial wilt has been reported as from the mid-1990-ies. The organism can still be found in local waterways. We hypothesized that the organism, which is of tropical and warm temperate origin, may have experienced conditions that are strongly selective for particular genotypic and/or phenotypic adaptations.

To investigate whether the Dutch *R. solanacearum* population has diversified, strains were isolated from water, bittersweet plants and sediment from a high risk area. These newly obtained strains (n = 42) were genetically and phenotypically analyzed and compared to two strains isolated from potato, i.e one Dutch and one tropical strain. Overall, the strains showed a clonal structure using a range of molecular methods. However, heterogeneity across strains was seen that appeared mostly related to recombination processes. We identified a 17.6 Kb putative genomic island (PGI-1), which was deleted from the genome of environmental strain KZR-5. Although the exact set of functions harbored by PGI-1 is as yet unknown, the island encompasses two genes of potential ecological relevance, i.e. one encoding a hypothetical protein with a RelA/SpoT domain and a putative cellobiohydrolase. We thus assessed the behavior of several strains under different environmental conditions.

Our results indicate that environmental strain KZR-5 has incurred altered fitness, with divergent outcomes, i.e. enhanced tolerance to cold stress versus reduced competitiveness during growth and in plant invasion. This also suggests a potential role for PGI-1 in the plant-invasive process. Three papers have so far resulted from this work. Patricia Stevens successfully defended her Ph.D. thesis on this topic in 2010.

The role of plasmids in bacterial adaptation to the mycosphere

In previous work on mycosphere-responsive bacterial communities, we found a plasmid in a *Variovorax paradoxus* –like responder. The plasmid was classed into the IncP1 group of broad host range (BHR) plasmids and was found to contain, next to its backbone, about 12 Kb of accessory sequence. We are on the track of determining the accessory load. Moreover, recent evidence has suggested that pasmids belonging to the prom2 group of BHR plasmids, exemplified by our rhizosphere-isolated plasmid pIPO2, also perform a role in mycosphere-associated bacteria.

This study aims to assess the dynamics and role of such mycosphere bacteria-associated plasmids. One hypothesis is that key adaptive yet dispensable genes are carried by the plasmid, whereas other thinking departs from the principle of plasmids behaving as the ultimate parasitic genetic agents. A Ph.D. student, Miao Zhizhang, was just hired on this project.

Monitoring of pathogenic microorganisms in water - development of a multipurpose, generic tool based on pathogenicity traits

The presence of pathogenic organisms in surface, ground and drinking water is a serious threat to the health of humans, animals and plants. Currently, water quality is mainly monitored by cultivation of indicator organisms, such as *Escherichia coli* and *Enterococci*. The use of indicator organisms may generate a false sense of security as the absence of indicator species does not necessarily correspond to the absence of actual

pathogens. Alternatively, detection of indicator species may overestimate the threat, resulting in unnecessary and expensive measures of control being taken. Thus, the aim of the project is to develop a monitoring tool based on cutting-edge insight into the genetic make-up and behavior of pathogenic organisms in water. The tool should allow for (high-throughput) detection of multiple pathogenic microorganisms in water systems.

For this purpose, we selected a functional characteristic (real pathogenicity trait) which is used by a range of animal and plant pathogens; a type III secretion system (T3SS). A large number of sequences that encode the conserved T3SS gene, *hrcR*, were collected from NCBI and used for comparison and primer development in conserved regions of the gene. (q) PCR systems which amplify the *hrcR* gene of pathogenic *E. coli, Shigella, Salmonella* as well as a number of plant-pathogens such as *Ralstonia solanacearum*, *Erwinia* spp. *Xanthomonas* spp. were developed and tested for specificity.

Fungal-associated bacterial communities and their adaptation

The selection of specific bacterial types in the mycosphere of the ectomycorrhizal fungus *Laccaria proxima* was investigated and extended to a laboratory situation. At two occasions (2004 and 2006), a consistent cluster of culturable bacteria was found to be selected in the mycosphere of the basidiomycetous fungus *Laccaria proxima* (*Agaricales, Tricholomataceae*) in the field. The bacteria, identified as related to *Variovorax paradoxus*, comprised 7.3 to 9% of the total culturable bacteria in the *L. proxima* mycosphere, but were not found in corresponding bulk soil (<0.3%). One strain, denoted HB44, was selected in order to unravel the basis of the *V. paradoxus* mycosphere competence in *in vitro* experiments with the former *L. laccata*, which was recently reclassified as *Lyophyllum* sp. strain Karsten (*Agaricales, Tricholomataceae*). In experiments in liquid culture, *L.* strain Karsten was shown to be an avid producer of glycerol, next to acetate and formiate (detected via proton nuclear magnetic resonance – NMR - analysis), which constituted the most abundant carbonaceous compounds released. Strain HB44 was able to grow avidly at the expense of the glycerol liberated by the fungus, as evidenced by proton NMR analysis of the fungal exudates in the medium before and after bacterial growth. In sterilized field soil, strain HB44 survived significantly better in the presence than in the absence of *L.* strain Karsten. Addition of a glycerol series to the sterilized soil (without the fungus) resulted in the persistence or growth of strain HB44, but only if the pH of this soil was previously set at 5.5. Thus, we provide evidence for the contention that tricholomataceous fungi can create specific niches in soil for the *V. paradoxus* related strain HB44 and that glycerol may be a main carbon source that drives the selection of this organism. This study made part of the thesis of F.G.H. Boersma, who successfully defended it in 2010.

Bacterial interactions with mycorrhizal fungi – migration via the fungal highway

Bacterial interactions with fungi are common in soil, and microscopic analyses have shown the existence of direct contact between bacterial and fungal cells. The mechanism behind these bonds of bacterial cells to fungal hyphae is often unknown. We hypothesized that soil bacteria, which live in a grossly carbon-deprived habitat, encounter novel ecological opportunities by the emergence of fruiting bodies of ectomycorrhizal fungi. In previous work, several bacterial groups that show a response to fruiting bodies have been identified in the mycosphere of *Laccaria proxima*. In this study, the selection of bacteria on the basis of their migration via fungal hyphae in soil was investigated in microcosm experiments containing *Lyophyllum karsten* (DSM2979). One week following inoculation with a bacterial community obtained from soil, selection of a few specific bacterial types was noticed at 30 mm in the growth direction of *L. karsten* in sterile soil. Cultivation-based analyses showed that the migration-proficient types encompassed ten bacterial groups, as evidenced by GTG-5 genomic fingerprinting as well as 16S rRNA gene sequencing. These were (>97% similarity) *Burkholderia terrae* BS001, *B. sordicola* BS021, *B. sedimenticola* BS010, *B. phenazinium* BS028, *Dyella japonica* BS013, BS018 and BS021, *Sphingoterrabacterium pocheensis* BS024, *S. daejeonensis* BS025 and *Ralstonia basilensis* BS017. Migration as single species was subsequently found for *B. terrae* BS001, *D. japonica* BS018 and BS021 and *R. basilensis* BS017. Typically, migration only occurred when these organisms were introduced at the fungal growth front, and only in the direction of hyphal growth. Migration proficiency showed a one-sided correlation with the presence of the *hrcR* gene, used as a marker for the Type III secretion system (TTSS), as all single-strain migrators were equipped with this system and most non-single-strain migrators were not. This stood in contrast with the low prevalence of TTSS within the bacterial community used as an inoculum (<3%). Microscopic examination of *B. terrae* BS001 in contact with *L. karsten* hyphae revealed the development of a biofilm surrounding the hyphae. Migration-proficient bacteria interacting with *L. karsten* may show complex behaviour (biofilm formation) at the fungal tip leading to their translocation and growth in novel microhabitats in soil. This study was carried out by R. Nazir, after early work together with Dr. J.A. Warmink.

Fungal-associated bacterial communities and the ecomolecular mechanisms involved

Basically, soil is a quite heterogeneous environment for the microbiota living in it. Among the soil inhabitants, bacteria and fungi are very important because they play essential roles in biogeochemical cycling. The carbon compounds released from the plants are key energy and nutrient sources for the soil microbiota. In the grossly carbon-limited soil, the emergence of plant roots and the formation of their associated mycorrhizae thus are

thought to create nutritional hot spots for soil-dwelling bacteria. As there is natural (fitness) selection in the soil, the bacteria which are best able to benefit from the hot spots have greatest probability to be selected. We are trying to understand the interactions of bacteria with soil fungi in these hot spots and to examine the key mechanisms involved in the selection of fungal-responsive bacteria.

We found evidence pointing to the fact that soil fungi modify the environment in their close vicinity by changing ecological factors like soil pH, thus creating suitable niches for specific bacteria. Furthermore, the provision of nutrients and the role of hyphae as dispersion vectors for bacteria are two other important fungal-based phenomena that enable these bacterial-fungal interactions. We have found that fungi can select specific bacteria over a range of dutch soils and there are some *Burkholderia terrae/hospita* - like (Bth) strains that are able to migrate along fungal hyphae in different soil types. One of the strains, encoded BS001, can migrate along with different fungal species. We also have preliminary data showing that strain BS001 is capable of protecting the fungus against antagonists in soil, probably by making a biofilm around the fungal hyphae or by degrading the antibiotics produced by fungal antagonists. Salient bacterial mechanisms that may be involved in these interactions are the efficient acquisition of specific released nutrients, the presence of type III secretion systems (TTSS), the capacity for flagellar movement and to form a biofilm, and – finally – the presence of plasmids as flexible carriers of genetic luggage. We have reviewed the available evidence supporting such mechanisms and this has resulted in a hallmark publication in 2009 (Nazir *et al.*). Recently we have sequenced the whole genome of bacterial strain BS001 *i.e.* approximately 11Mbp. Two copies of the TTSS were shown to be present. We expect to understand bacterial-fungal interactions in a better way by digging into this huge genome in the near future.

Microbial community ecology – diversity and community niche

Research on biodiversity and ecosystem functioning takes many angles, from the diversity and stability of communities to the preservation of ecosystems and ecosystem services. In spite of the overwhelming microbial diversity, the concern about how environmental changes affect life in our planet has also taken microscopic scales. It is therefore crucial to understand how ecosystem processes are affected by microbial diversity, but also the mechanisms through which microbial diversity affects community functioning. These are key questions in the research carried out by the tenure tracker Joana Falcão Salles involved in this novel line of research.

Predicting biodiversity effects on ecosystem functioning requires adequate evaluation of the mechanisms explaining why more diverse systems could perform better than less diverse ones. In this context, tackling functional diversity has become an important issue. By using experimental communities of denitrifying bacterial species, Salles et al (2009) showed that by defining an index for the breadth of the niche occupied by a community, one should be able to predict ecosystem processes which are related to resource extraction by that community in a given multiple resource environment. The experiment consisted of determining the activities of 16

bacterial species grown individually on six different carbon sources. Then, the same set of species was used to assemble communities varying in both species richness and composition in microcosms containing a mixture of all six carbon sources. The performances of individual species on individual carbon sources were then used to calculate an a priori index called ''community niche'' that accounted for the performances of the species present in a given community across the entire range of the six resources. Salles et al (2009) showed that bacterial species richness had a positive but small effect on community processes whereas community niche explained a much larger proportion of the variation (fig.1). Thus, by aggregating resource-use characteristics of species in a community into a synthetic descriptor of community niche, one is able to better predict the observed variations in community functioning. In addition to community niche, the presence of particular bacterial species also influenced community functioning, indicating that other effects than the capacity to use the carbon sources provided in culture medium played a role in the experiment. This study demonstrated the importance of complementarity for resource use among species in explaining the enhanced performance of diverse bacterial communities.

Microbial diversity and invasiveness

Biodiversity is implicated in the likelihood of a community to be invaded by alien species. Theoretical and experimental studies have indicated that biologically-diverse plant communities are often less prone to being invaded than simpler ones, but effects of microbial diversity on invading organisms have remained unexplored. Previous experiments in our laboratory have supported an inverse relationship between an invading species, *E. coli* O157:H7, and the diversity of the microbial community it encounters. These experiments have also hinted to the idea that at least one of the mechanisms behind this inverse relationship is resource complimentarity. This year we were able to show that in experimental single niche systems composed of one carbon source more diverse bacterial communities are able to use more resources, and at a faster rate, than less diverse communities. In the context of invasion, this high rate and consumption of resources limits the dominance of *E. coli* in a microbial community.

Expanding on this idea that resource use limits an invader's access to resources, we plan to test this hypothesis with experimental soil microcosms. We will use two approaches to test this hypothesis: an assemblage and dilution-to-extinction approach. In the assemblage approach, we will inoculate and follow *E. coli*'s survival in soil communities composed of 1 to 80 strains. In the dilution-to-extinction approach, we will dilute soil microbial communities in a stepwise fashion to obtain communities of different diversities and then follow *E. coli*'s survival after inoculation. This will allow us to examine resource use and invasion at much higher levels of species richness than the assemblage counterpart. In both these approaches, we will measure community resource utilization and expect that higher diverse communities will extract more resources than less diverse communities, thus aiding to *E. coli*'s decline.

The microbiology of bird eggs

A collaborative project with animal ecology (Prof. dr. Irene Tieleman) aims to explore how environmental conditions shape variation in microbial communities and to determine the evolutionary consequences of these changes for the protective systems of birds against microorganisms. Because of the complexity of the avian immunity, an 'egg-nest' dyad represents a simple model system to study the association between microbial communities on egg shells and antimicrobial defence proteins into albumen. Until now, the project has been focused on both microbial and immune methodology. Further experimental approaches will aim (1) to experimentally manipulate the microbial community in nests and to look at the effects on antimicrobial proteins of eggs, in a captive bird (zebra finches, *Taeniopygia guttata*), and (2) to perform a comparative study on eggs of two tropical bird species across a range of environmental conditions in Kenya.

Bacterial endophyte communities of arctic plants are cold-adapted and host plant specific

Plants in all climates and in all ecosystems are associated with a diverse array of microbes. Endophytic bacteria inhabit the internal plant tissues, and have been isolated from a large diversity of plants, where they form non-pathogenic, often mutualistic, relationships with their hosts. Although the presence of endophytic bacteria has been known for long, awareness of their abundance and potential benefits has grown rapidly in recent years. The reported plant beneficial traits include growth stimulation, nutrient mobilization, nitrogen fixation, detoxification of pollutants and biocontrol of plant pathogens. There are only a handful of studies on diversity of endophytic bacteria in natural ecosystems, and currently there is no data on endophytic bacterial diversity in arctic flora.

Arctic and subarctic climates are highly demanding environments for plants, due to low temperature average and extreme cold, short growth season (1–4 mo) and low solar radiation, with high annual fluctuations. Water stress, either in the form of drought or flooding, is common. As recycling of nutrients, especially nitrogen and phosphorus, in cold climates is very slow, the soil is often very nutrient-poor. To survive in this highly demanding environment, arctic plants employ diverse anatomical and physiological adaptations like low-growth morphology, dominantly vegetative propagation, low optimal temperature for photosynthesis and physiological hardening to prevent frost damage.

The study characterized the taxonomic and functional diversity of endobacterial flora of three arcto-alpine plant species (*Oxyria digyna, Diapensia lapponica* and *Juncus trifidus*) in the low Arctic in Kilpisjärvi area, northwestern Scandinavia (69°03'N). The endobacterial flora was isolated from plant samples collected from three distinct sites, and endophyte community diversity was analyzed by cultivation and DNA-based methods. Functional diversity of culturable endobacteria was analyzed by activity assays and by PCR amplification of functional genes.

Analysis of bacterial endophyte library of

over 350 isolates as well as 6 clone libraries each encompassing 90- sequences revealed a high diversity of bacteria living in association with arctic plant species, representing Actinobacteria, Bacteroides, Firmicutes, Acidobacteria and α- , β- and γ- proteobacteria. The most common taxonomic groups were α- and β- proteobacteria and Actinobacteria (30.6%, 26% and 27.4%, respectively). Taxonomic distribution of the culturable isolates as well as the clone libraries were dependent mainly on host plant species, but also on (properties of) sampling sites (Figure 1).

Several bacterial groups associated tightly with specific plant species: *Burkholderia* spp. dominated *D. lapponica* and *J. trifidus* samples, but were nearly absent from *O. digyna*. *Sphingomonas* spp. were common in *D. lapponica* as well as in *O. digyna*, and sequence alignment of sphingobacterial isolates revealed their plant host specificity. Moreover, *Sphingomonas* spp. from different plant hosts showed divergent metabolic profiles.

The isolated bacteria were well adapted to low temperatures; this is reflected in both taxonomy, with the closest relatives often representing psychrophilic isolates, as well as in their physiology: most isolates grow well at +4°C. Phosphate solubilization is a very common trait in isolates analyzed, as well as production of ACC deaminase. In contrast, cellulase or amylase activity were detected in less than 10% of the analyzed isolates. Many of the isolates retained full enzymatic activity at +2–+5°C.

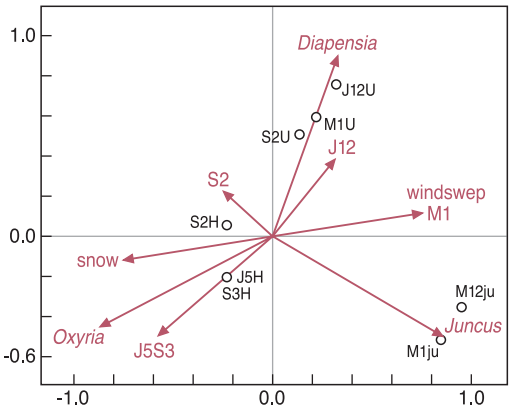

Figure 1. Simple ordination plot of taxonomic distribution of endophyte isolates from arctic plant species. The first letter and number in sample codes indicate sampling site, whereas the last letter indicates sampled plant species; U= *Diapensia lapponica*, Ju= *Juncus trifidus*, H= *Oxyria digyna*.

Ecology of Bacterial Endophytes in Sustainable Agriculture – Rice root-associated bacteria

Plants are the major source of carbohydrates for the heterotrophic microorganisms on Earth. For their growth, the latter organisms thus rely heavily on the efficient production of photoassimilates by plants. Plants even make use of diverse compounds to interact, and form associations, with often mutualistic beneficial bacteria. On the other hand, bacteria possess a wide range of metabolic properties that may modulate plant growth. Bacteria living inside plants, i.e. bacterial endophytes, might intimately interact with cells of the host, taking up secreted metabolites and releasing plant-growth-promoting compounds. This synergistic interaction has been recently demonstrated and exemplifies a so-called double-fitness trait which is active in the plant-endophyte partnership. P.R. Hardoim, R.M. Nissinen and J.D. van Elsas recently reviewed this area in the context of novel findings therein.

In one study by P.R. Hardoim, the effects

of plant genotype, soil type and nutrient use efficiency on the composition of different bacterial communities associated with rice roots were investigated. Thus, total bacteria, Alpha- and Betaproteobacteria, Pseudomonas and Actinobacteria were studied using PCR, followed by denaturing gradient gel electrophoresis (PCR-DGGE). Rice genotype determined, to a large extent, the composition of the different bacterial communities across cultivars. Several cultivars belonging to *Oryza sativa* ssp. indica tended to select similar bacterial communities, whereas those belonging to subspecies *japonica* and aromatica selected ones with divergent community structures. An effect of soil type was pronounced for the Actinobacteria communities, while a small effect of 'improved' and 'traditional' plants was noted for all communities analyzed. A few dominant bands in PCR-DGGE, affiliated with *Rhizobium radiobacter*, *Dickeya zeae, Mycobacterium bolletii* and with members of the Rhizobiales, Rhodospirillaceae and Paenibacillaceae, were spread across cultivars. In contrast, a majority of bands (e.g. affiliated with *Enterobacter cloacae* or *Burkholderia kururiensis*) was only present in particular cultivars or was erratically distributed among rice replicates. These findings suggested that both bacterial adaptation and plant genotype contribute to the shaping of the dynamic bacterial communities associated with roots of rice plants (Hardoim *et al*., 2011).

Mining of ecosystems for novel bacterial chitin degrading enzymes – abundance and diversity of chitinases in well known and unexplored habitats

Chitin is the second (after cellulose) most abundant biopolymer on Earth. Although chitin is not produced by bacteria, it is used by them as a source of nitrogen and carbon, or of energy. The majority of bacterial chitinases is assigned to family 18 of the glycosyl hydrolases, in particular to type A encoded by *chiA* types of genes.

Investigation of different habitats with respect to their "chitinase pool" is important to understand the ecology of this function among the microbial populations and communities and to establish future plans for their use. The main objective of present study was to obtain new information about chitin degraders and chitinases with improved characteristics. Thus, the chitin degradation potential of the microbial communities in ten different terrestrial and aquatic habitats was assessed.

Degradation of oligomeric substrates and fluorimetric quantification was used for evaluation of total chitinolytic activity. Genetic analysis based on *chiA* PCR-DGGE and deep sequencing (454) were performed for assessing the abundance and diversity of the bacterial chitinolytic communities. In order to correlate the total diversity of *chiA* with the active bacterial community, a qPCR approach was established. Additionally, the effect of chitin enrichment on the dynamics of the chitin-responsive microbial communities was studied in soil microcosms, in which particular conditions such as pH and chitin enrichment were set.

Clear differences were observed in the abundance of *chi* genes in different habitats. We linked the results of enzymatic chitinase assays with molecular data focusing on a comparison of the diversity and abundance of

chiA genes in the selected environments. We found divergent patterns of richness/diversity versus function. Our study provides new insights in ecosystem composition in terms of the diversity and function of bacterial chitinases.

The functional and structural prokaryote diversity of soil under potato

This project aims to elucidate the functional and structural prokaryote diversity of soil under potato in order to understand how the plant affects the microbiota associated with it. Three out of four years were used for sampling fields laid out with different potato cultivars in a randomized design. The experiments were executed in the field and the full growth season (third of four years) was monitored. The first season sampling was completed, including rhizosphere and bulk soil samples take at three plant growth stages. As a result of the work on soil DNA extraction, there was a publication "*Effect of soil DNA extraction method on the apparent microbial diversity of soil*" published in Applied and Environmental Microbiology. A second paper "*Effect of plant genotype and growth stage on the ß-proteobacterial community associated with different potato cultivars in two fields*" was also published in Applied and Environmental Microbiology.

Pyrosequencing analysis was done for the first year samples. Indicator organisms such as several members of the *Betaproteobacteria* were suggested to indicate soil quality. Links between oligotrophs and copiotrophs were also shown. The results were liad down in a paper submitted to Plos ONE. Furthermore, a quantitative PCR system to target the *asfA* gene (sulfur mineralization process) was optimized. A microcosm experiment was set up to see the effect of sulfonate on the soil microbial community, particularly on desulfonators and betaproteobacterial community. Since there is no naturally available sulfonate compound, sodium dodecyl benzenesulfonate was used. We measured the concentration of the sulfonated compound in soil in collaborative work with a FR group. First of all, the growth ability of *Variovorax paradoxus* with sulfonated compounds in liquid medium was assessed to determine if the organism uses this compound as sulfur and/or organic source. Afterwards, a greenhouse experiment was set up with two potato cultivars (high and low starch content) and different concentration of sulfonate.

Besides, fluctuations in the bacterial community over the three growth seasons in the bulk soil on one field were analyzed by DGGE, qPCR and clone libraries. Additionally, the BIOLOG phenotype array was used to see specific aspects of soil microbiological characteristics and represents an independent analysis of differences or changes in soil community structures and functions for the last sampling moment.

The baseline of soil functioning across a range of soils

Soil microorganisms play key roles in several important ecosystem processes, including nutrient acquisition, nitrogen cycling, carbon cycling and soil formation. Thus, the microorganisms in soil are important in maintaining the quality of agricultural soil systems. It is known that there is a natural fluctuation of the soil microbiota and its activity, which is caused by factors such as soil type and soil edaphic factors, plant type, soil management practices and environmental perturbations.

Agricultural ecosystems annually receive approximately 25% of global nitrogen input, mostly in the form of ammonium, which must be oxidized at least once to nitrate by a nitrifying community, and biological nitrogen fixation lies at the basis of all life on Earth. Therefore, the understanding of these essential processes of the nitrogen cycle is of great agronomical and environmental importance. Hence, this project will, through an in-depth analysis of a representative number of soils selected all over the Netherlands, evaluate the baseline of soil functioning and the impact of soil abiotic conditions and season, using different nitrogen cycling genes and several microbial groups as bioindicators.

In 2010, we continued with soil sampling and based our analyses on directly-extracted soil DNA. Specifically, we focused on 8 soils sampled across the Netherlands in April, June and October. We used PCR-DGGE fingerprinting to study the diversity of archaeal *amoA* (AOA), bacterial *amoA* (AOB) and nitrogen-fixing bacteria (*nifH*). We found that all these communities are affected by the different sampling times and by soil texture/pH. We also found that the communities are affected by pH, but also clay content, especially AOA and AOB. To detect and quantify several important genes in our soils, we used quantitative PCR for the following genes: *nifH* (nitrogen fixation), AOA (ammonia oxidizing archaea) and AOB (ammonia oxidizing bacteria). The average copy number per gram of soil for the different groups, in logarithmical scale, varied from 5.0 to 7.0 for all genes analyzed: *nifH*, AOA *amoA* and AOB *amoA*. We also measured nitrification and denitrification activities of the 8 soils, and we found higher values of both activities in clayey soils, compared with the sandy soils. A significant correlation between archaeal ammonia oxidizers and nitrification activity was found, but not with bacterial ammonia oxidizers.

Up to now, there are no appropriate mathematical methods applied in biology which can satisfactorily address the baseline variation in soil. Several statistical as well as modeling approaches were tested for the possible characterization of the NOR of the selected eight Dutch soils. The approach which is described below was selected as the best in term of the quality of received results, accuracy, as well as possible applicability by the end users. This will be achieved by taking into account all the soil and microbial measurements simultaneously. The NOR can be considered as a space of n dimensions, where n is the number of variables measured (at the present time 22 parameters are used for the calculations, resulting in a space with 22 dimensions). When the soil is not disturbed, all combinations of the variables fall in the "normal operating range". The distance between a certain state and the center of the NOR will represent a quantitative measurement that summarizes the state of the soil, taking into account the multivariate nature of the data. The approach is a type of multivariate analysis which takes into account all soil characteristics which have been measured so

far. One of the advantages of the approach is that the data themselves will show which variables contribute the most to the NOR and which produce noise.

The parameterization of the model was done by performing microcosm experiments as well as by sampling selected soils in natural conditions. In order to characterize short-term variations and the influence of different stress factors on the changes in total microbial communities in a regular agricultural soil, three microcosm experiments were carried out (two experiments in Groningen and one in Utrecht). To distinguish the natural variability in field conditions, soil sampling and analyses of eight soils across the Netherlands have been done regularly every three months for at least two years.

Estimating the baseline of the autochthonous microbial community and nutrient fluxes in soils will be a sensitive method that distinguish the influence of possible soil disturbances (e.g. usage of GMO) on general soil health. Moreover, the method can assist in the distinguishing of the critical parameters in soil which are out of NOR as well as in the prevention of unnecessary changes. Finally, knowing the biochemical soil characteristics and those procedures that lead to a more stable and balanced soil would directly point to ways to preserve soil health and stability.

Culturing the 'unculturables'; a challenging approach to recover new species from the rhizosphere - Ecology of *Acidobacteria* and *Verrucomicrobia*

The ecology of bacterial groups that are known for their recalcitrance to growth in culture media, such as the *Acidobacteria* and *Verrucomicrobia*, was studied. Information about the distribution of these groups in rhizosphere and bulk soil is scarcely available and occasionally even conflicting reports were made about their distribution over the different plant-soil compartments. Culturable representatives of both groups obtained from leek and potato rhizospheres were used to study their ecological behaviour in different plant-soil compartments.

Two isolates from the *Acidobacteria* group 8 and nine from the *Verrucomicrobia* subdivision 1 were recovered from oligotrophic agar media either or not amended with catalase or root exudates and incubated at 4% CO_2 after plating of rhizophere extracts. Their potential to interact with plants was studied *in vitro*. Three approaches were followed to determine possible interactions: (1) growth on different carbon sources common in plant root exudates (*i.e.*, organic acids, aminoacids and sugars); (2) *in vitro* studies on growth effects in sterilized plants (3) differences in distribution over plant-soil compartments using real-time PCR with primer systems specific for the isolates.

Both *Acidobacteria* (CHC25 and ORAC) were able to utilize malate, succinate and glutamine. The *Verrucomicrobia* isolates were able to utilize two or more of the following compounds: oxalate, malate, succinate, citrate, glutamine and/or alanine. Isolate CHC8, belonging to the *Verrucomicrobia* subdivision 1, was the only isolate that was able to degrade cellulose. In the *in vitro* assays with sterilized plants, it was demonstrated that the isolates influenced plant growth differently. Isolate CHC12 (*Verrucomicrobia*) inhibited the growth of 20% of the inoculated leek plants,

and the sizes of these plants were reduced by approximately 50%, in comparison with the uninoculated leek plants. Verucomicrobial isolate ONA9 colonized the root surface of leek plants, but did not influence shoot or root growth. Both *Acidobacteria* isolates increased the root size of leek plants by about 40% in comparison with uninoculated plants. Quantification of *Verrucomicrobia* isolates introduced into the plant-soil system revealed a higher abundance in the rhizosphere (about 10^6 cell per gram of soil) than in the bulk soil (10^4–10^5 cell per gram of soil). Numbers of introduced *Acidobacteria* group 8 isolates (determined by real time PCR) were grossly the same in rhizosphere and bulk soils.

This study provided clear indications that some of the *Acidobacteria* and *Verrucomicrobia* isolates interact with plants. The role of these isolates in plant growth promotion and rhizosphere functioning will be further explored in plant-soil microcosm and field studies. Ulisses Nunes da Rocha successfully defended his PhD on this topic in October of 2010.

Publications 2010

Doctorate granted by the institution, prepared within the institution

Nunes da Rocha, U., 2010. *Ecology of Acidobacteria and Verrucomicrobia in plant-soil ecosystems*. Rijksuniversiteit Groningen. Promotor.: Prof. dr. Ir. J.D. van Elsas.

Stevens, P., 2010. *Adaptation of Ralstonia solanacearum biovar 2 to temperate climates*. Rijksuniversiteit Groningen. Promotor: Prof. dr. Ir. J.D. van Elsas.

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Theoretical Biology

Group leader Prof. dr. F.J. Weissing

Composition of the group in 2009:

Tenured Staff	**source**	**fte**	**period**
Prof. dr. R. Bijlsma	RUG	1.0	
Prof. dr. C.K. Hemelrijk	RUG	1.0	
Prof. dr. I.R. Pen	RUG	1.0	
Prof. dr. F.J. Weissing	RUG	1.0	
Post-docs			
Dr. H.L. Dugdale	NWO-Rubicon	0.5	091201-101201
Dr. L.M. Engqvist	RUG	1.0	081001-111001
Dr. H. Hildenbrandt	RUG Rosalind Franklin	0.5	100915-111015
Dr. T.A. Revilla-Rimbach	NWO	1.0	090701-100801
Dr. P. van den Berg	NWO-ERGO	1.0	101101-130501
PhD Students			
Drs. R.F. Castillo Cajas	EU Marie Curie	1.0	090101-130101
Drs. A.L.F. Duarte	RUG	1.0	061101-110301
Drs. A.B.F. Ivens	RUG	1.0	061001-111101
Drs. A.L.W. Kuijper	RUG	1.0	060701-100701
Drs. L. Molleman	UE Bursaal	1.0	091001-130930
Drs. J.E. Oosten	RUG Rosalind Franklin	0.8	070501-100701
Drs. I. Puga-Gonzalez	RUG	1.0	080701-120701
Drs. L. Ross	RUG	1.0	061001-101001
Drs. E.E. van der Vaart	NWO-TopTalent	1.0	070915-110915
Other PhD Students			
Drs. P. Girod	University of Zürich		
Drs. H. Kunz	University of Zürich		
Administrative Staff			
secretary			
H.G. Tjoelker	RUG	0.5	090901-100901
I.C. Jansen	RUG	0.4	100901-110901
Technical Staff			
ICT-Counsellor			
J. Bakker	RUG	1.0	

Introduction

Most of the models developed in the Theoretical Biology group address research questions in ecology, evolution, and behavioural biology. We see it as one of our main tasks to bridge the gap between theoretical and empirical research. The group intends to contribute to this task in three ways. First, our models are framed in terms of 'concrete' variables and parameters with a clear biological meaning. In other words, the models are more 'mechanistic' and less phenomenological than is standard in theoretical research. Second, we strive to include slightly more complexity in the models than is typical for the 'toy models' that are often used to illustrate conceptual ideas. Overly complex models do not inspire much insight, while overly simplistic models may be misleading when applied to particular real-world situations. With models of intermediate complexity, we hope to achieve a good balance between conceptual simplicity and analytical tractability on the one hand, and realism and testability on the other. Third, the model predictions are systematically confronted with real-world situations. In close collaboration with colleagues from various biological disciplines, the group tries to test its model predictions both in the lab and in the field. With the modelling approach indicated above, we address questions in three interrelated research areas:

Adaptive variation

Variation and diversity are key concepts in the biological sciences. Much of the variation found in nature is the result of stochastic processes like mutation, recombination, genetic drift, and environmental or demographic stochasticity. However, at all levels of biological organisation part of the variation is structured in a systematic way. Most probably, much of this patterned variation results from directional processes like competition or selection. It is one of our core aims to investigate how such 'adaptive variation' does arise and how it is maintained on an ecological or evolutionary time scale. At the behavioural level, we are interested in the evolution of 'animal personalities', the phenomenon that individuals differ systematically in whole suites of behavioural tendencies, and that, moreover, these tendencies are stable in time and correlated across contexts. How can various behavioural types coexist; why isn't there a single type of highest fitness that outcompetes all other types? And why are behavioural tendencies stable in time and consistent across contexts; shouldn't one expect a more flexible structure of behaviour? At the life-history level, we are interested in the evolution of alternative phenotypes, like the differentiation of a primordially homogeneous population into distinct morphs, distinct mating types or distinct castes of workers. Under which circumstances should one expect the evolution of coexisting specialist phenotypes, rather than a single generalist phenotype? Why, for example, is the evolutionary transition from a state where all individuals are simultaneous hermaphrodites to a population with two separate sexes common in animals, but rare in plants? What are the evolutionary and ecological consequences of morph differentiation? What are, for example, the evolutionary and ecological implications of the interplay between natural and sexual selection? At the population level, we are interested in the emergence of new species. To what extent can speciation be viewed as an adaptive process? Can we predict speciation on the basis of intraspecific and environmental factors? How can our understanding of speciation be used to explain global biodiversity patterns? At the community level, we are interested in species coexistence and the interaction of ecology and evolution in shaping com-

munity patterns. Why can hundreds of algal species coexist in a droplet of water while the 'principle of competitive exclusion' predicts that the number of coexisting species will not exceed the number of limiting resources? To what extent are the structure of a food web and the energy flow pattern within a food web the result of adaptive evolution?
Interestingly, the answer to one type of question at one level of organisation is often of a similar structure as the answer to a quite different question at a different level of organisation. Indeed, many explanations of adaptive variation can be traced back to two basic principles: evolutionary branching resulting from architectural constraints and non-equilibrium dynamics caused by non-transitive interactions as in the Rock-Scissors-Paper game. Based on this general insight, there is hope that the detailed study of seemingly diverse examples of adaptive variation may in the end result in a general theory for the emergence and stability of biodiversity patterns.

Cooperation and conflict

The history of life on earth can be seen as a sequence of major transitions in which lower-level entities evolve to cooperate with each other and form ever higher levels of biological organisation. Genes cooperate to form genomes and cells, cells cooperate to form multicellular individuals, individual organisms cooperate to form social communities and species cooperate in mutualistic interactions. Yet the stability of higher-level cooperative units is constantly threatened by potential conflict between lower-level entities over their share in the genetic contribution to future generations. We study how this balance between conflict and cooperation shapes individual development, the social structure of populations and the dynamics of mutualistic interactions.
A fundamental developmental process is sex determination. Will an individual develop into a female, a male or both and become a hermaphrodite? What cues are used to trigger sexual development? We build models to investigate how the evolution of sex determination is affected by parent-offspring conflict, sexual conflict and environmental variability. A great advantage of studying sex determination is that model predictions are relatively easy to test by observing sex ratios in the lab or in the field. We collaborate with various national and international partners to test our models in a wide range of taxa, from tiny short-lived hermaphroditic worms to long-lived reptiles. More generally, we develop models to study the role of maternal effects and epigenetics on the evolution of individual developmental plasticity. To what extent should an individual 'listen' to cues transmitted by its mother or by the environment? What are the evolutionary implications of epigenetically mediated transgenerational inheritance?
Highly social species, such as humans and eusocial insects, are characterized by strong division of labour. We study under what conditions division of labour can 'self-organize' in groups of phenotypically plastic individuals. How can development of discrete casts evolve from an initially homogeneous workforce? How important is cultural evolution as opposed to genetic evolution? Mutualistic interactions between species are common and ecologically important. Yet it is still far from clear how mutualisms remain stable in view of the temptation to exploit partners. What mechanisms are expected to evolve to ensure stable cooperation? How important are partner choice and sanctions against non-cooperative partners? What is the role of horizontal versus vertical transmission of partnership?

Self-organization of social systems

In many biological systems, the understanding of patterns and processes is enhanced by the explicit consideration of different levels of organization. Seemingly well-understood phenomena at a higher level of organization can often in a surprising way be re-interpreted as emergent properties from the interactions among units at a lower level. This may be helpful in gaining understanding of both the mechanisms of group behaviour and their evolution. For instance, empirical validation in cooperation with empirical scientists shows that the typical complex patterns of flocking of huge swarms of thousands of starlings at their roosting site only appear in a model of 'fish schooling' if we add banking while turning. Because the flocking behaviour of massive starling 'clouds' is difficult to study empirically, such models are needed to gain better understanding of its mechanistic properties. Further, in a model of groups of individuals that perform dominance interactions and show affiliate behaviour, a difference in a single parameter (intensity of aggression) affects the social structure in such a way that the society switches from the many characteristics of a despotic society to that of an egalitarian society. Thus, complex differences between societies may be caused by a difference in a single trait at a lower level, and the evolution of social systems may, correspondingly, reflect the evolution of a few individual traits. By applying evolutionary principles to these lower-level processes, those patterns and processes will be singled out that are evolutionarily stable. The integration of an evolutionary and a self-organization approach is a major challenge and one of the central goals of our research programme.

Overview of academic results

Although we had to move again in 2010, this time from the Biological Centre to the brand new Life Sciences building at the Zernike Campus, our group continued at a stable pace in 2010. Charlotte Hemelrijk presented her inaugural lecture "Slim of niet? - Cognitie of zelforganisatie" in June. Tomás Revilla and Laura Ross successfully defended their thesis and are now continuing their scientific careers as post-docs at the Central University of Venezuela at Caracas and the University of Oxford (UK), respectively. Bram Kuijper and Ana Duarte will defend their thesis in 2011, but did already start with a post-doctoral position in two different research groups at the University of Cambridge (UK). Piet van den Berg started as a post-doc in our group to work on the ERGO project of the Dutch Science Organisation (NWO) on the potential spread of genetically modified crop genes. Franjo Weissing was main applicant for a 4 M€ EU grant for an international Erasmus Mundus Joint Master's Programme "Evolutionary Biology" (MEME; www.evobio.eu) that is coordinated by the University of Groningen and organized together with the universities of Montpellier (France), Munich (Germany), Uppsala (Sweden) and Harvard (USA). Together with Bert Groen (UMC Groningen), he was also main applicant for a 4 M€ NWO grant for a Systems Biology Centre "Energy Metabolism and Ageing" (SBC-EMA) at the University of Groningen. Franjo Weissing does now combine research and teaching with his functions as Dean of Research at the Faculty of Science, director of the Groningen Graduate School of Science, and co-ordinator of the Master's programme MEME. In 2010, Ido Pen

organized the symposium "Genetic Conflicts: Engines of Evolution?" Our group is looking forward to the organisation of the bi-annual meeting Mathematical Models in Ecology and Evolution (MMEE; www.mmee2011.evobio.eu) in 2011, which for the first time will take place outside the UK.

Concerning publications, there were several highlights. Most notably, Ido Pen was lead author of a *Nature* article on the effects of climate on sex-determination, and Franjo Weissing is co-author of a *Science* article (to appear in 2011) on the evolution of pattern formation in mussel beds. Both articles will be briefly summarized below. Kuke Bijlsma was co-editor of a special issue of *Conservation Genetics* on "Integrating Population Genetics and Conservation Biology".

The following contributions give an impression of the work being done in the Theoretical Biology group. Franjo Weissing gives an overview of our recent work on speciation, the evolution of animal personalities, and the interplay between sex allocation and sexual selection. Bram Kuijper shows how sexual selection operates in hermaphroditic versus gonochoristic species. Laura Ross summarizes some results from her thesis on "Genetic conflict and sex allocation in scale insects". Leif Engqvist presents some results on the evolution of male fertility. Aniek Ivens describes her work on the mutualism between ants and root aphids. Last but not least, Daan Reid presents some highlights of his work on hydrodynamic models of fish schools.

Is speciation adaptive?

Franjo Weissing (in collaboration with Sander van Doorn, University of Bern, Switzerland; Pim Edelaar, CSIC, Sevilla, Spain)

Speciation – the origin of new species – is the source of the diversity of life. A theory of speciation is essential to link poorly understood macro-evolutionary processes, such as the origin of biodiversity and adaptive radiation, to well understood micro-evolutionary processes, such as allele frequency change due to natural or sexual selection. An important question is whether, and to what extent, the process of speciation is 'adaptive', *i.e.*, driven by natural and/or sexual selection. In his seminal book on the *Origin of Species by Natural Selection*, Darwin (1859) envisaged speciation as the result of two processes: selection for diversification allowing the exploitation of previously unused opportunities, and the extinction of intermediate forms as a consequence of severe competition among these forms. Hence, according to Darwin selection plays a major role in the speciation process. However, Darwin's verbal arguments are often vague and not always convincing, partly because of his pre-Mendelian ideas on inheritance. It is partly for this reason that the founding fathers of the "Modern Synthesis" largely discarded Darwin's view on speciation, giving non-selective factors like geographic isolation and the accumulation of potentially deleterious mutations by genetic drift a much more prominent role than selection.

In the last decade, the view that adaptive speciation is a rare and unlikely phenomenon has been challenged by two recent developments in speciation theory, which seem to suggest that natural and sexual selection can be more powerful in the creation of new species than the traditional models seem to

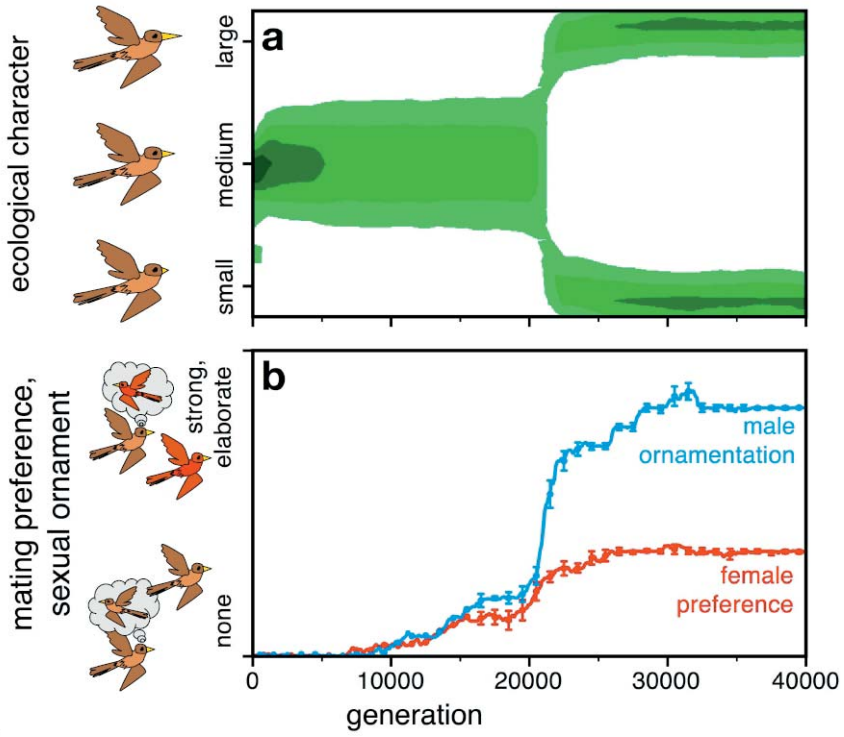

Figure 1. Adaptive speciation by the joint action of natural and sexual selection. In the absence of sexual selection, disruptive selection for ecological specialisation will typically not lead to speciation, but to a broad distribution of ecological characters (bill sizes). The variation in ecological characters, many of which not well adapted to local circumstances, creates favourable conditions for the spread of costly female preferences for a costly condition-dependent male ornament (plumage colour). Once this preference has evolved, natural and sexual selection mutually reinforce each other, eventually leading to reproductively isolated ecological specialist species.

suggest. First, a new class of 'ecological' models of speciation selection models convincingly demonstrates how frequency dependent selection can lead to the evolution of ecological differentiation. Second, 'sexual selection' models of speciation show how divergent sexual selection can lead to the diversification of mating strategies and, hence. However, taken on their own, both modelling approaches are not fully convincing. Ecological models can explain the stable coexistence of incipient daughter species in the face of interspecific competition, but they are typically vague about the evolution of reproductive isolation. Sexual selection models can explain the evolution of prezygotic reproductive isolation, but they are typically vague on questions like ecological coexistence. By means of integrated models, incorporating both ecological interactions and sexual selection, we demonstrated that disruptive selection on both ecological and mating strategies is necessary, but not sufficient, for speciation to occur. To achieve speciation, mating must at least partly reflect ecological characteristics.

In an invited review paper (Weissing, Edelaar & Van Doorn, *BES*, 2011), we argue that the current tendency to view natural and sexual selection as alternative and contrasting mechanisms underlying speciation is counterproductive and misleading. In fact,

adaptive speciation can most easily be achieved when natural and sexual selection work in concert. This is exemplified in a recent model (Van Doorn, Edelaar & Weissing, *Science*, 2009) that shows how natural and sexual selection can mutually reinforce each other, thereby achieving adaptive speciation under conditions where speciation would have been considered highly unlikely by classical theory (Figure 1).

The evolution of spatial pattern formation

Franjo Weissing (in collaboration with Monique de Jager, Johan van de Koppel, Peter Herman, and Bart Nolet, Netherlands Institute of Ecology, Yerseke and Wageningen, The Netherlands)

When searching for food, shelter and other resources animals face the daunting complexity of the natural world, where resource distributions are often quite unpredictable. To cope with the challenge to maximize the probability of resource encounters, many organisms adopt specialized search strategies. Brownian and Lévy walks are prominent examples of random walk strategies where both the direction and step length of the constituent moves are drawn from a probability distribution. In case of Lévy walks, step lengths follow a power-law distribution (with a characteristic exponent μ), where many short steps are occasionally alternated with a long step. Several theoretical studies suggest that a Lévy walk with exponent $\mu \approx 2$ is the most efficient random search strategy in resource-limited environments where food occurs patchily at locations unknown to the searcher. Although shown to be optimal for only these specific conditions, Lévy walks with $\mu \approx 2$ are broadly found in nature, suggesting that they are actually adaptive over a wider range of conditions. In a recent study (De Jager *et al.*, *Science*, in press), we hypothesize that this wide occurrence is due to the fact that resource distributions are often not completely determined by external factors, but also by the organisms consuming or otherwise using these resources. This feedback between resource and organism distribution leads to an intriguing interplay between ecology and evolution: natural selection should lead to movement strategies that an optimally adapted to the patterning of the environment; but the environment in turn is shaped by the movement strategies of the organisms.

We illustrate these general considerations by a concrete example that is amenable to experimental investigation. On intertidal flats, the distribution of regularly-spaced clumps of mussels (*Mytilus edulis*) results from the interaction between local mussel density and the crawling movement of young mussels. Pattern formation in mussel beds is attributable to two opposing mechanisms: cooperation and competition. Through movement into cooperative aggregations, mussels increase their local density, thereby decreasing wave stress and predation risk. Conversely, competition for algae, which occurs on a larger spatial scale than facilitation, prevents the formation of larger clumps by limiting the number of mussels within a long range. The interaction of local facilitation and long-range competition results in the emergence of a patchy distribution of individuals, which simultaneously reduces risk and minimizes competition for algae. The distribution patterns found in mussel beds are readily reproduced in lab experiments (Figure 2C). Investigating the movement patterns of individual mussels shows that – also in this system –

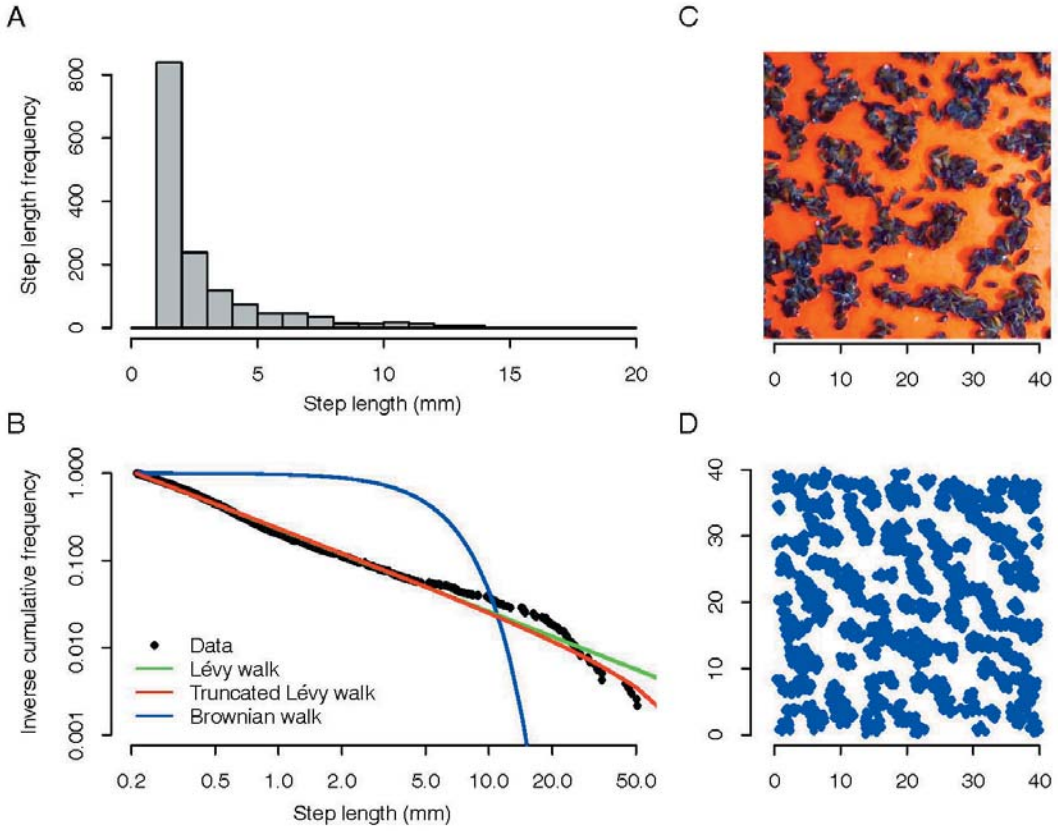

Figure 2. Pattern formation by mussel movement strategies – comparison of experimental and modelling results. (A) In lab experiments, solitary mussels (*Mytilus edulis*) show a movement pattern with a characteristic 'step length' distribution. (B) This movement pattern is best described by a (truncated) Lévy walk. (C) In the experiments, the movement patterns of many mussels results in a characteristic distribution of the mussels over space. (D) A similar pattern results in individual-based simulations when the moving individuals follow a Lévy movement strategy. Moreover, such a strategy is the only evolutionarily stable strategy.

the search strategies of mussels corresponds to a Lévy walk with exponent $\mu \approx 2$ ($\mu = 2.1$ provides the best fit; Figure 2AB). Individual-based simulations confirm that such a search strategy does indeed result in the spatial patterns characteristic for mussel beds (Figure 2D).

An evolutionary analysis based on adaptive dynamics methods revealed why a Lévy walk strategy with $\mu = 2.1$ is superior to other movement strategies. To this end, we considered a wide spectrum of movement strategies. For each strategy m, we considered the 'resident' population consisting of individuals all exhibiting strategy m. For each such strategy, the individual movements will result in a characteristic pattern $P(m)$ (= a characteristic spatial distribution of mussels). Given this pattern, we could then calculate the reproductive success ('fitness') of all alternative strategies m'. Here we assumed that reproductive success is dependent on the energy spent moving and the energy gained by food consumption (reflecting local competition with other mussels). The resulting fitness function $F(m',m)$ could be analyzed by the

methods of adaptive dynamics. It turned out that the only evolutionarily stable and convergence stable strategy was a Lévy walk with exponent $\mu = 2.1$, as found in our experiments. Interestingly, this exponent is larger than the value $\mu = 2.0$, which leads to a spatial pattern of mussels that is optimal in the sense that it maximizes the average fitness of the population. Hence, evolution does not optimize the 'performance' of a mussel bed as a whole. From an evolutionary perspective, this is not surprising, since natural selection should maximize individual fitness rather than population fitness. The spatial pattern of a mussel bed may be viewed as a 'public good'. A mussel bed generated by the resident strategy $\mu = 2.0$ is 'vulnerable' in the sense that the resident strategy can be 'exploited' by mussels with a strategy $\mu > 2.0$. Only the (ecologically sub-optimal) strategy $\mu = 2.1$ cannot be exploited by alternative strategies and is, hence, evolutionary stable.

This is one of the first examples demonstrating how large-scale spatial patterns may result from the interplay of ecology and evolution.

The evolution of animal 'personalities'

Franjo Weissing (in collaboration with Max Wolf, MPI Human Development, Berlin, Germany; Sander van Doorn, University of Bern, Switzerland)

In many animal species, ranging from squids to chimpanzees, individuals of the same sex, age and size differ consistently in whole suites of correlated behavioural tendencies, comparable to human personalities. Various 'behavioural types' coexist within the same population, and such types exhibit behavioural consistency over time (*e.g.*, types that are more aggressive as juveniles are also more aggressive as adults) and across contexts (*e.g.*, types that are more aggressive towards conspecifics are also bolder towards predators). Both types of consistency indicate limited behavioural plasticity to a degree that, from an adaptive point of view, is often surprising. Shouldn't a more flexible structure of behaviour provide a selective advantage? On a proximate level, behavioural consistency can often be understood in terms of the architecture of behaviour, that is, the genetic, physiological, neurobiological and cognitive systems underlying behaviour. But the question remains why architectural constraints on the flexibility of behaviour have not been removed by natural selection, and why not a single behavioural type outcompetes all other types in the population.

We developed a series of conceptual evolutionary models to address these questions. With these models we aim to address aspects of animal 'personalities' that appear to have some universality (*i.e.*, occur in a range of animal species). In a first attempt (Wolf *et al.*, *Nature*, 2007), we showed that individuals differing in future fitness expectations (*e.g.*, individuals having territories of different quality) should systematically differ in their risk-taking behaviour. In short, individuals with high fitness expectations (having 'much to lose') should be more risk-averse in all kinds of contexts than individuals with low fitness expectations. This 'asset protection principle' does not only provide an explanation for the commonly observed boldness-aggressiveness syndrome, but also allows to make *a priori* predictions on the 'architecture' of risk-related behaviour. Recently, our predictions were confirmed in experiments with

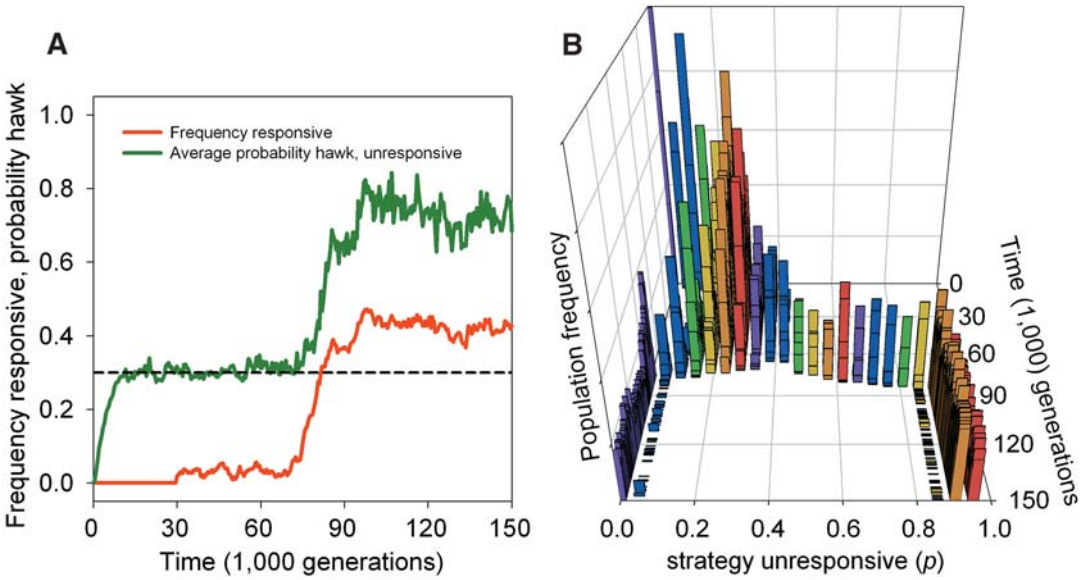

Figure 3. Co-evolution of social sensitivity and behavioural consistency in the hawk-dove game. Individual-based simulations where, after 30.000 generations, we allow rare mutations that give rise to responsive individuals. (A) Responsive individuals increase in frequency over time (red line) and select for an increased frequency of hawks among unresponsive individuals (green line). (B) The presence of responsive individuals gives rise to benefits of consistency which, in turn, select for high levels of consistency among unresponsive individuals, that is, unresponsive strategies with maximal behavioural consistency (p = 0 or p = 1) are favoured over less consistent ones.

various organisms (including insects, fish, birds and mammals).

In a second model (Wolf *et al.*, *PNAS*, 2008), we provided an explanation for the common observation that individuals often differ along a responsiveness (reactivity, flexibility, awareness) axis: at the unresponsive end of this spectrum we find individuals that quickly develop routines and show relatively rigid behaviour; at the responsive end we find individuals with a more extrinsic control of behaviour; these individuals are more flexible and readily respond to environmental stimuli. We could demonstrate that selection on responsiveness is negatively frequency dependent. This explains the evolutionary emergence of behavioural types differing in responsiveness. However, our model also partially explained why individuals should be consistent in their responsiveness over time and across contexts.

This aspect is address in a third model (Wolf *et al.*, *Proc. R. Soc. B*, 2011), which studies responsiveness in the context of social interactions (also called 'social sensitivity'). We could show (Figure 3) that consistent differences in responsiveness can evolve through a co-evolutionary feedback process. The model reveals that responsive strategies can get off the ground whenever there is sufficient variation in the population (*e.g.*, due to mutation and drift), since responsive individuals can exploit small deviations from equilibrium. Subsequently, the presence of responsive individuals triggers the evolution of behavioural consistency in unresponsive individuals, since – in two-strategy games with a mixed-strategy ESS – consistent individuals are exploited to a lesser extent than inconsistent ones. Behavioural consistency makes individuals predictable and, hence, favours responsiveness. In other words, there is a positive feed-

back between behavioural consistency (and predictability) and social sensitivity. We currently investigate the implications of this feedback for the evolution of cooperation.

In an invited review (Wolf & Weissing, *Phil. Trans. R. Soc. B*, 2010), we made an inventory of the most important evolutionary mechanisms leading to individual variation in behaviour and to consistency of behaviour, both in time and across contexts. In a second invited review (Wolf & Weissing, *Trends Ecol. Evol.*, in press), we discuss the ecological and evolutionary implications of individual variation in behaviour.

The evolution of communication strategies

Franjo Weissing & Ido Pen (in collaboration with Jan Komdeur, Animal Ecology Group; Carlos Botero, NESCent, Durham, USA)

Unexpectedly, individual variation also plays a central role in a different project, where we modelled the evolution of communication strategies. Communication is a process in which senders provide information via signals and receivers respond accordingly. The evolution of signalling has already been modelled repeatedly, but implicitly all these models assume *a priori* that the evolving signals have a 'meaning' for their receiver. In contrast, we developed a model where a signalling strategy (a 'sender code' determining what kind of signal is to be sent given the sender's state) co-evolves with a response strategy (a 'receiver code' determining the response of the receiver to different types of signal). In our model, sender and receiver codes are represented by evolvable norms of reaction.

Our model (Botero *et al.*, *Evolution*, 2010) is based on a situation where individuals signal their 'quality' (more specifically, their fighting ability) in agonistic interactions with rivals. Each individual is repeatedly confronted with rivals and must repeatedly choose whether to attack or not. Individuals differ in quality and in case of escalation the opponent of higher quality has a higher probability of winning the fight. In a situation like this, signalling quality may be profitable because it can help avoid costly escalation. This will only happen if (1) senders do not 'cheat', that is, if signal intensity shows a clear-cut relationship with individual quality; and if (2) receivers respond adequately by avoided escalated fights with individuals signalling high quality. When talking about signals, we imagine the badges of status of birds. Accordingly, we use the terms 'signal intensity' and 'badge size' interchangeably.

In our individual-based simulations, communication strategies always converged rapidly to equilibrium. As expected from costly signalling theory, the average sender code evolves into a norm of reaction where individuals of higher quality produce larger badges. The average receiver code at equilibrium is a norm of reaction where the probability of attack increases with own quality but decreases with the size of the opponent's badge. Surprisingly, however, the *average* norms of reaction are not representative for individual behaviour. Upon inspection of the individual distribution of traits in the evolved populations, we discovered that individual behaviours were strikingly different from the average population codes. To explore the nature of this variation we summarized each sender code with the average badge size produced by this code and each receiver code with its average probability of attack. Figure 4

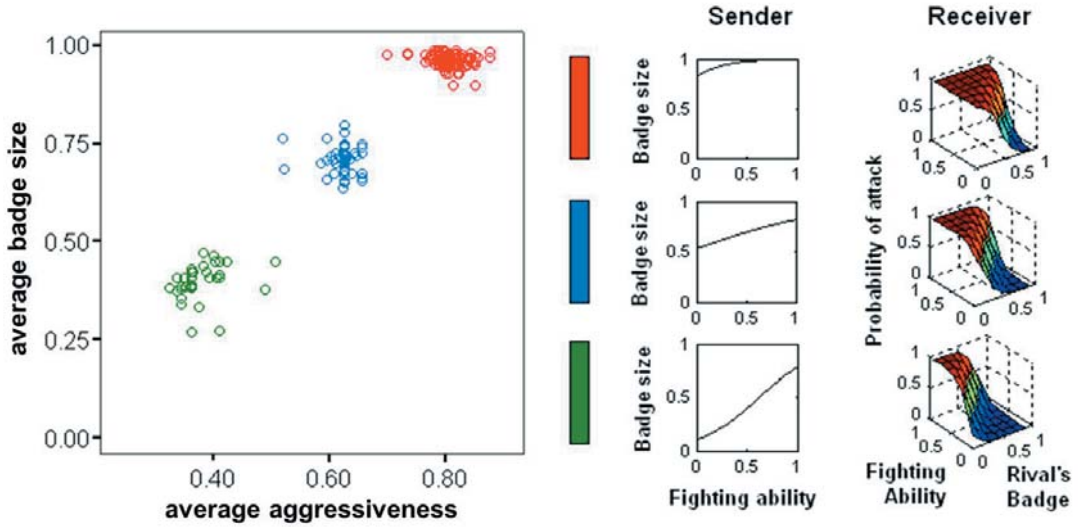

Figure 4. Individual variation in communication strategies predicted by our model of badges of status. To the left, we represent the sender and receiver strategies of all individuals of an evolved population by the average signal intensity ('badge size'; *y*-axis) and the average response elicited ('aggressiveness'; *x*-axis). A cluster analysis reveals that there are three distinct combinations of sender and receiver strategy in the population, which are indicated by the panels to the right.

(left panel) depicts these summary statistics for each of the 3,000 individuals at the end of a representative simulation run. The figure reveals that there are three distinct co-existing strategies in the population that are represented to the right in Figure 4. One class of individuals (green) develops relatively small badges and is readily 'intimidated' by opponents with large badges. A second class of individuals (red) develops large badges irrespective of underlying quality and is highly aggressive irrespective of the signals received from opponents. A third class of individuals (blue) is of an intermediate type.

A closer analysis of the model suggests that alternative communication strategies (alternative 'dialects' of a common language) will evolve whenever senders possess imperfect information about their own quality or state. These findings provide an explanation for recent reports of individual differences in communication strategies. They also shed some new light on the evolutionary stability of 'cheating': while reliable information about the signallers' quality is conveyed within each type of communication strategy, misrepresentation and misinterpretation occurs across communication strategies. Last, but not least, our model provides a new kind of explanation for the coexistence of behavioural types (animal 'personalities'): if individuals differ in the production and interpretation of signals, they will also show consistent differences in behaviour.

The evolution of self-organized division of labour

Ana Duarte, Franjo Weissing & Ido Pen (in collaboration with Laurent Keller, University of Lausanne, Switzerland)

Division of labour is a key determinant of the ecological success of eusocial insects. However, the seemingly harmonious functioning of a colony with often thousands of individuals is not well understood. Self-organization models demonstrate that division of labour at the colony level may be an emergent property of the interaction of many agents, each of which being guided by relatively simple behavioural rules. These models may provide a proximate explanation for division of labour, but they lack an evolutionary perspective. Conversely, evolutionary models focus on the relationship between division of labour and colony fitness, thereby neglecting the behavioural mechanisms underlying division of labour. In a recent review (Duarte *et al.*, *Ann. Rev. Ecol. Evol. Syst.*, in press), we plead for an integration of both approaches. In a nutshell, we consider organisms with a simple but relatively open behavioural architecture (like a neural network). The characteristics of this architecture (e.g., the number and type of neurons, the connections among neurons and their properties) are heritable properties, which are subject to mutation and natural selection. The behaviour of each individual is governed by its inherited architecture, and the joint behaviour of all individuals in the colony determines (via self-organized division of labour) the reproductive success of the colony. This way, successful architectures, that is, architectures leading to well-coordinated colony behaviour will be selected.

We have applied this approach in various models that have led to some interesting insights. First of all, we could show that self-organized division of labour can indeed evolve from scratch, that is, from a genetically and phenotypically undifferentiated population. This is illustrated by Figure 5, which is based on the "fixed threshold model", *the* textbook example for self-organized division of labour. In the original self-organization model, there are – from the start – two types of workers with different thresholds for two different tasks. It is not too surprising that the workers with a low threshold for task 1 specialized on performing task 1, while the workers with a

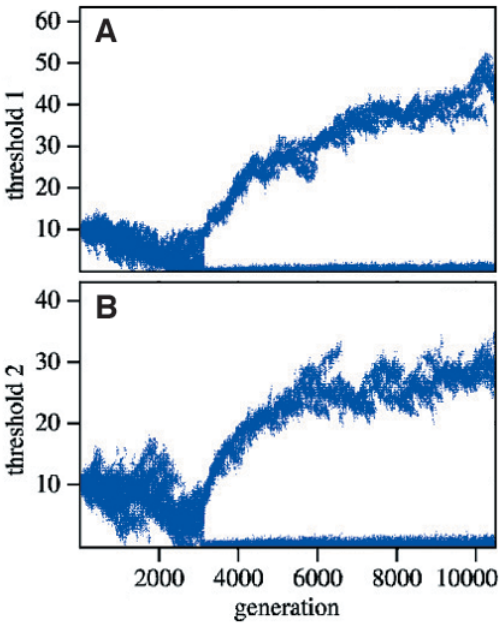

Figure 5. Evolution of thresholds for two tasks in the "fixed threshold model" for self-organized division of labour. Initially, all workers had the same threshold (=10) for both tasks. First, both thresholds evolve to lower values, until around generation 3000 threshold differentiation occurs. In the end, part of the population has a low threshold (=0) for task 1 and a high threshold (=30) for task 2, while the remainder of the population has a high threshold (=40) for task 1 and a low threshold (=0) for task 2. The resulting population exhibits adaptive division of labour.

low threshold for task 2 specialized on task 2. The question is how the differentiation in thresholds occurred in the first place. Figure 5 demonstrates that disruptive selection can lead to threshold differentiation and, thus, division of labour, in an initially undifferentiated population. Our second insight was that the situation in Figure 5 is far from representative. Threshold differentiation will only occur under highly specific circumstances. It is more representative that all thresholds converge to zero, thus undermining the threshold mechanism. Third, the 'bauplan' underlying the behavioural architecture matters a lot. For example, self-organized division of labour evolves much more easily in a neural network model than in a threshold model. However, the evolved networks have some strange properties. Moreover, the evolutionary outcome is not robust in the sense that small changes in the model assumptions (*e.g.*, the way task performance affects colony fitness) may have major effects on the types of networks that evolve. All in all, we have to conclude that the evolution of self-organized division of labour is not yet well understood.

Extending the theory of sexual selection

Bram Kuijper, Ido Pen & Franjo Weissing (in collaboration with Ellen van Velzen, COCON; Lukas Schärer, University of Basel, Switzerland)

Sexual selection is a powerful evolutionary force that underlies the evolution of exaggerated male characters, such as horns and other weapons used in male-male competition for access to females, or conspicuous songs and displays used to attract and persuade females into mating. Importantly, sexual selection results from a co-evolutionary interplay between the sexes: male ornaments evolve to match a female's preference, and a female preference in turn evolves as a function of the variation in male phenotypes. This evolutionary interdependence of male and female characters makes it difficult to make verbal predictions. In such a case, formal models are an ideal tool to generate logically consistent hypotheses. In a recent review (Kuijper, Pen & Weissing, *Ann. Rev. Ecol. Evol. Syst.*, in press), we tallied more than 150 recent models on the co-evolution between the sexes.

Still, many aspects of sexual selection are not yet well understood. For example, many seemingly general predictions of sexual selection theory are based on the implicit assumption that male and female traits will converge to equilibrium. However, co-evolutionary processes like sexual selection – which in many respects resembles an arms race between the sexes – do often not converge. As shown by Van Doorn & Weissing (*Am. Nat.* 2006), such non-equilibrium dynamics can have major implications, *e.g.*, on the diversity and the information content of sexual signals.

Moreover, there are many areas where sexual selection theory needs to be extended. For example, mutual mate choice or cryptic female choice have not yet received much attention by modellers. One field with major gaps in our knowledge is sexual selection in hermaphrodites. Indeed, virtually all sexual selection models were developed for species with two separate sexes. This is surprising, since hermaphroditism is the dominant mode of reproduction in plants, while in animals (where about 6% of all species are hermaphroditic) some of the most spectacular examples of sexual conflict have been found in hermaphrodites. We therefore developed a

variety of sexual selection models that were specifically tailored to (simultaneous) hermaphrodites.

It is easy to realize that not all results of standard sexual selection theory can directly be extrapolated to hermaphrodites. First,

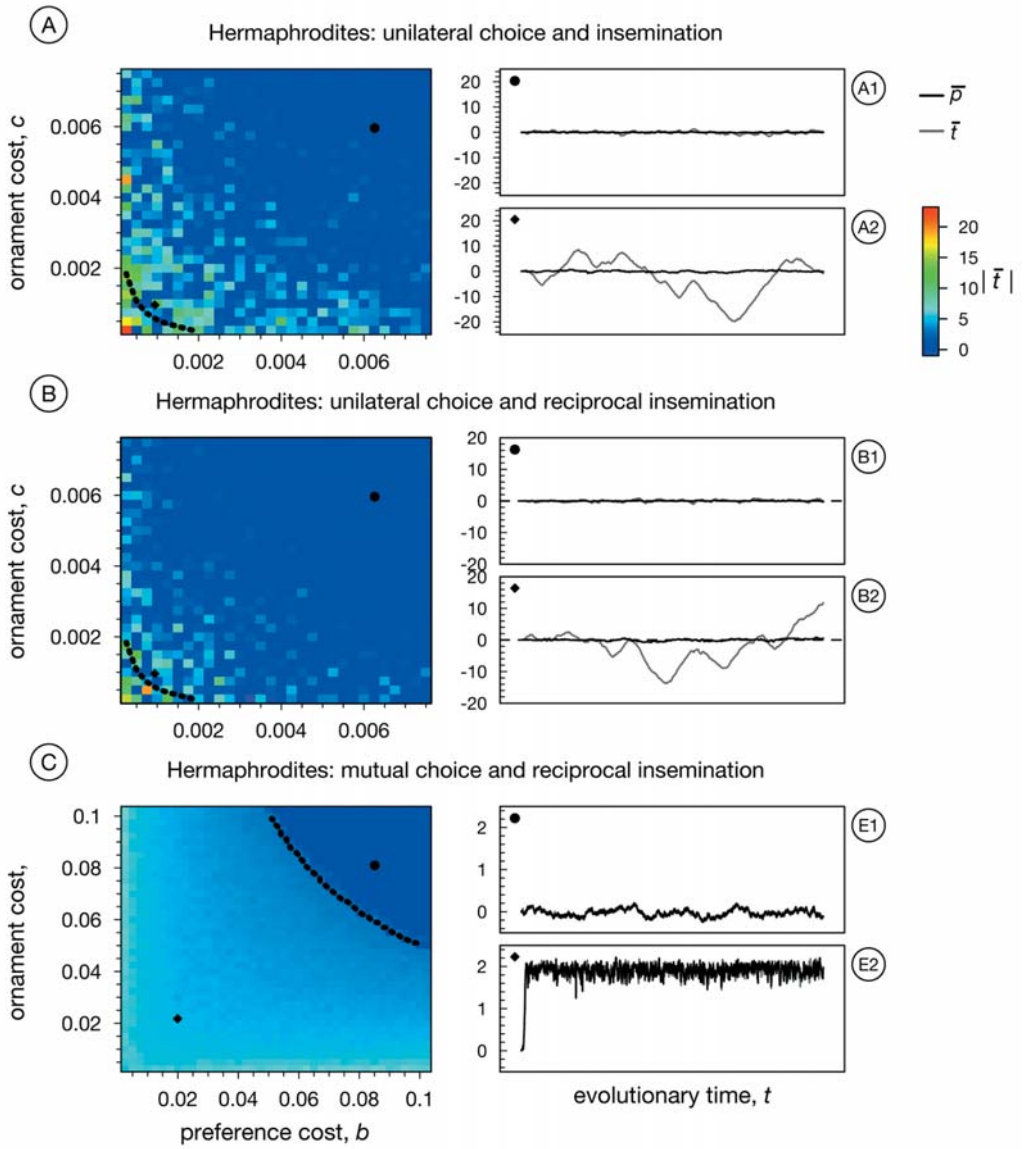

Figure 6. Sexual selection in hermaphrodites. For three scenarios of mate choice and mating patterns, the panels to the right illustrate the outcome of individual-based simulations for a given combination of cost parameters. The region below the dashed line corresponds to those parameter combinations where our analytical model predicts that costly preferences for a costly signal should evolve. The panels to the right show the level of preference (p) and ornament (t) that evolved in six example runs, corresponding to the squares in the panel to the right. Sexual selection gets most easily off the ground if mating reflects the choice of both partners (mutual mate choice). Yet, a maximal level of ornament expression (t) is achieved in species with unilateral choice. The direction of insemination (unilateral *versus* reciprocal) has only a marginal effect on the strength of sexual selection.

standard theory assumes that the expression of sexual ornaments is sex-limited, implying that only males have to bear the costs of such ornaments. Since such sex-linkage is impossible in (simultaneous) hermaphrodites, the costs of ornamentation are higher than in species with two separate sexes. Second, standard theory assumes standard sex roles, with one choosy sex and the other sex competing for being chosen. In hermaphrodites, the complications of mutual mate choice cannot be avoided. Third, selection on males and females often acts in opposing directions. For example, males are typically selected to realize as many matings as possible, while a much smaller number of matings is optimal for females. In hermaphrodites, this sexual conflict translates into a genetic conflict between the male- and female-function genes of one and the same individual. Fourth, sexual selection in hermaphrodites is intricately related to sex allocation, *i.e.*, to the relative investment into male and female function. In a first model (Van Velzen, Schärer & Pen, *Proc. R. Soc. B*, 2009), we studied the interaction of sex allocation and sperm competition. We showed, for example, that the mechanism of cryptic 'female' choice can have important implications for the outcome of evolution and that sexual conflict in hermaphrodites can trigger the evolution of separate sexes (more precisely, into males and into hermaphrodites with female-biased sex allocation).

By constructing models in a systematic and step-by-step fashion and by comparing the outcome of individual-based simulations with analytical predictions, we are building up an inventory of sexual selection models allowing us to evaluate relative importance of ingredients like reproductive mode (hermaphrodites versus gonochorists), mating system (*e.g.*, unilateral versus mutual mate choice), or timing of mate choice (pre- *versus* post-copulatory). This is illustrated by Figure 6, which shows the evolution of costly preferences in hermaphrodites. While sexual selection gets most easily off the ground in case of mutual mate choice, the strength of sexual selection (indicated by the degree of evolved ornamentation) is highest in case of unilateral mate choice. The outcome is hardly affected by the directionality of insemination. Comparing this figure with a comparable figure for gonochorists leads to the conclusion that – at least in the case of mutual mate choice – sexual selection may well be as strong in hermaphrodites as in organisms with separate sexes; something regarded as impossible by many evolutionary biologists, including Charles Darwin. Moreover, our study also demonstrates that simple models of sexual selection are not very robust when it comes to particularities of how individuals choose their mates.

Sex ratio control erodes sexual selection

Bram Kuijper, Ido Pen & Franjo Weissing (in collaboration with Tim Fawcett, University of Bristol, UK)

How much animals should invest in sons and daughters is a central problem in behavioural ecology. In 1973, Bob Trivers and Dan Willard suggested that parents should bias offspring sex ratios in relation to certain environmental factors, if those factors influence the fitness of sons and daughters differently. One influential idea is that females mated to attractive partners should overproduce sons, since these sons will inherit their father's attractiveness and so enjoy high mating success. This has been investigated mainly in birds, where

males typically have elaborate courtship displays and females, with two different sex chromosomes, potentially have some control over offspring sex. But while some studies have reported a link between male attractiveness and brood sex ratios, others have found no such relationship. In the past, we developed theory to achieve a better understanding of these experimental results. We could show that the above-mentioned verbal arguments are generally correct, but that the relationship between sex ratio bias and father's attractiveness should reflect the genetic architecture of the sexually selected traits.

In a recent article (Fawcett *et al.*, *PNAS*, in press) we add another twist to the story. This is illustrated by Figure 7. We performed individual-based simulations for various standard models of sexual selection. As shown in the initial time period in Figure 7 (up to the dashed line), female preferences and male ornamentation evolve to the theoretically predicted equilibrium. In the initial period, the sex ratio was fixed at 0.5, but from this point onwards, it was allowed to evolve. As shown in the figure, a strong sex ratio bias evolves where females mated to attractive partners indeed overproduce sons, while females mated to less attractive males overproduce daughters. Intriguingly, the sex ratio bias has strong repercussions on sexual selection, that is, on the very same process driving its evolution. As shown in the upper panel of Figure 7, the evolved sex ratio bias leads to a reduction in both female preferences and male ornamentation.

Two main processes are responsible for the erosion of sexual selection by sex-ratio control. First, sex-ratio adjustment allows females with unattractive partners to mitigate the fitness disadvantage of low male ornamentation. In simple terms, ending up with an unattractive male is not so disastrous if a female can skew offspring production towards daughters. This reduces the fitness benefit of female choosiness. Second, since choosier females tend to mate with more ornamented males and therefore produce mainly sons, their strong preference genes will rarely be expressed by their offspring. This lowers the average female preference in subsequent generations and thereby reduces the fitness benefit of male ornamentation.

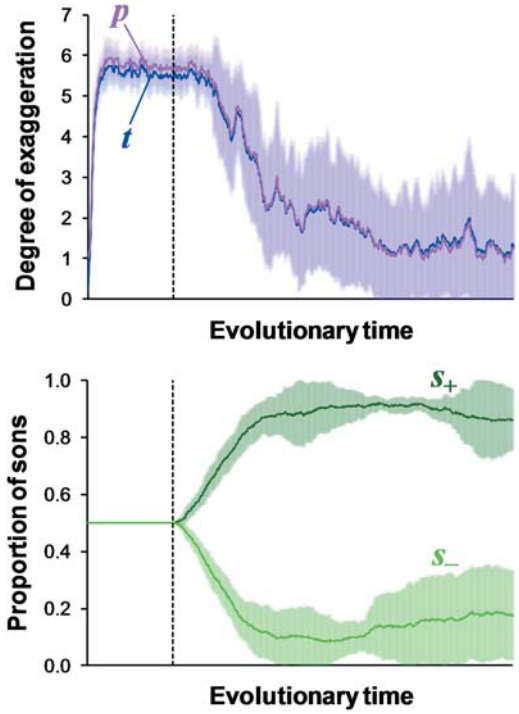

Figure 7. Sexual selection and sex allocation interact in a dynamic fashion. The upper panel shows the level of male ornamentation (t, blue) and female preference (p, pink), while the lower panel shows the female sex-allocation strategies s_+ and s_-, where s_+ (dark green) is the probability of producing a son when her partner has above-average ornamentation and s_- (light green) is the probability of producing a son when he has below-average ornamentation. Offspring sex ratios are initially unbiased ($s_+ = s_- = 0.5$), but are allowed to evolve from the point indicated by the vertical dashed line. The divergence of s_+ and s_- shows that a strong sex ratio bias evolves where females mated with attractive males mainly produce sons, while females mated with unattractive males mainly produce daughters. This sex ratio bias in turn erodes sexual selection: as seen in the upper panel, male ornamentation and female preferences converge to much lower levels.

Consequently, the equilibrium levels of female preference and male ornamentation are substantially lower when sex-ratio adjustment is possible. In effect, sex ratio control undermines the very process that selected for it in the first place. Our model predicts that the most elaborate ornamentation should be seen in species with little control over the sex ratio.

By means of a simple but generic analytical model, we could demonstrate that the outcome of our individual-based simulations is robust and general. Moreover, the feedback described above points to a more general evolutionary principle, in which a conditional strategy weakens directional selection on another trait by reducing fitness differences.

Climate-driven population divergence in sex-determining systems

Ido Pen, Barbara Feldmeyer & Anna Harts (in collaboration with Tobias Uller, University of Oxford, UK; Geoffrey While and Erik Wapstra, University of Tasmania, Hobart, Australia)

Sex determination is a fundamental developmental process, yet its mechanisms are remarkably diverse. Vertebrates exhibit both genotypic (GSD) and temperature-dependent sex determination (TSD). The latter is particularly common in reptiles and both systems can co-occur within taxonomic families. In addition, some species show elements of both genotypic and environmental sex determination within populations. Explaining the causes behind repeated evolutionary shifts between GSD and TSD and the origin and maintenance of mixed systems are two of the greatest unsolved problems in sex determination research. The two main reasons why the diversity in reptilian sex determination has remained an enigma have been (1) a failure to empirically link incubation temperature to ecological conditions promoting TSD and (2) to establish theoretically that those conditions are sufficient to drive evolutionary shifts in sex-determining systems. In a joint theoretical and empirical effort, we set out to tackle these problems (Pen *et al.*, *Nature,* 2010).

A lizard, the snow skink *Niveoscincus occelatus*, seemed ideally suited for our purpose. This species has populations with GSD that live in the harsh climate of the Tasmanian highlands, and populations with TSD in the more temperate coastal areas of the island. Very few species are known where GSD and TSD coexist, and even fewer with well-studied life history and ecology. Fortunately, much was known about the snow skink's life history and how changes in thermal conditions affect the fitness of females and males. This allowed us to construct mechanistic models for the evolution of sex determination, and to parameterize these models with data of a well-studied species.

Theoretically, whether TSD is selectively favoured over GSD depends on the balance of two opposing forces. On the one hand, sex differences in the fitness consequences of timing of birth could favour integration of temperature-dependent developmental processes and gonad differentiation to ensure a match between offspring sex and birth date. On the other hand, sufficiently strong temperature fluctuations between years select against TSD, since years with extreme temperatures would cause very biased sex ratios in a TSD population, giving the stable sex ratios guaranteed by GSD the upper hand.

We constructed detailed models of the sex-specific life histories of both highland and lowland snow skink populations, and in combination with field data from many years we used these to estimate how birth date affects fitness of males and females. It turned out that male fitness was hardly affected by birth date, for both populations, but for females there was a clear advantage of being born early in the lowland population, while no or hardly such advantage was present in the highland population. This implies a selective advantage for TSD in the lowland population, but not in the highland population. However, in both populations, temperatures fluctuated considerably between years, albeit more strongly so in the highland. This tends to select against TSD, so the question remained what the net selective effect on TSD vs. GSD was. To answer this question required detailed evolutionary models, integrating both aspects.

Some simulation results are shown in Figure 8. The model generated two primary results, both in close accordance with empirical data. First, in simulations parameterized with data from the lowland population, sex determination evolved from pure GSD to-

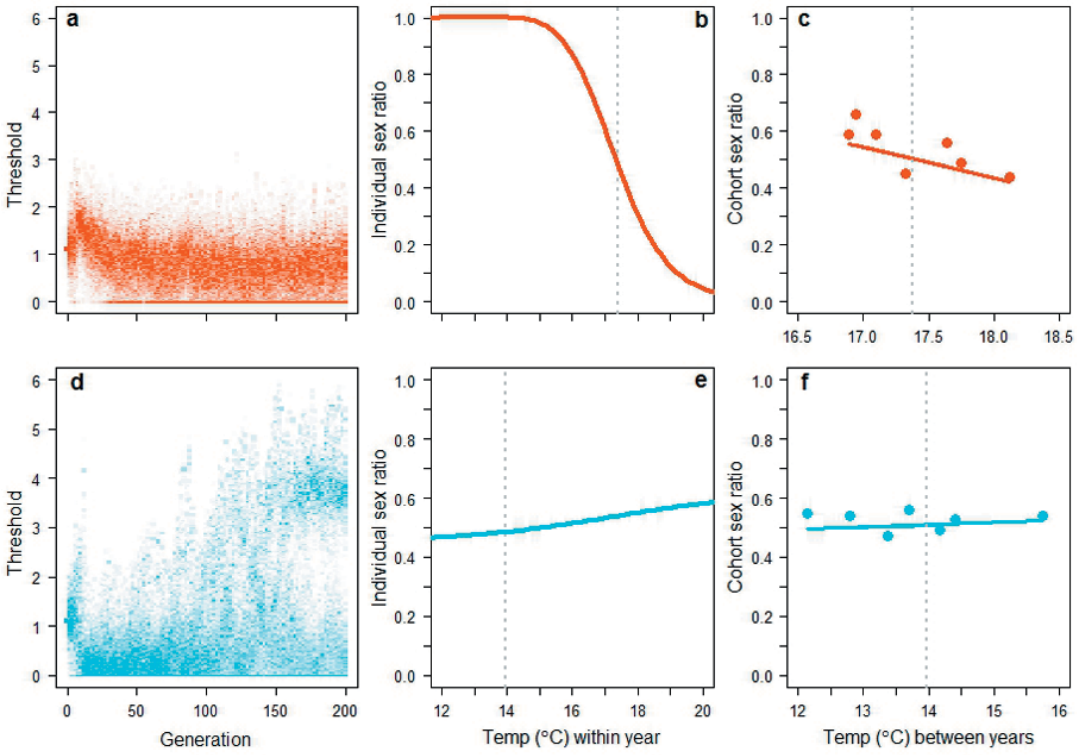

Figure 8. Individual-based simulations of the evolution of sex-determining mechanisms. The parameterization of the model was based on snow skink data. Simulations were initialized with genetic sex determination as ancestral state. Upper panels for lowland parameter settings and lower panels for highland parameter settings. **a**/**d**. Population distributions of allelic values at threshold locus changing of time. Note branching for highland parameter settings, resulting in a novel sex-determining locus: males 'homozygous' for alleles causing low thresholds and females 'heterozygous' for low and high threshold alleles. **b**/**e**. Evolved average reaction norm for offspring sex ratio as a function of developmental temperature. Vertical dotted line average temperature experienced by natural populations. **c**/**f**. Predicted (from evolved reaction norm; line) and observed (natural populations; squares) cohort sex ratios for annual mean maximum temperature in the wild.

wards a system with a strong temperature effect (Figure 8b). This generated a significant negative correlation between the cohort sex ratio and average temperature during gestation that closely resembled data from the natural population (Figure 8c). Second, in simulations parameterized with data from the highland population, sex chromosomes of the initial GSD system were either retained or, if lost, were replaced by a novel genetic element of major effect via disruptive selection (Figure 8d). Consequently, the model could generate evolutionary shifts from one sex chromosome system to another – including transitions between male and female heterogamety - but it always produced a sex-determining system that generated average sex ratios that did not deviate substantially from equality, again in close accordance with the natural population (Figure 8e,f). These results were robust with respect to starting settings, male versus female heterogamety, and linkage between genetic elements

Climate driven population divergence in sex-determining systems emphasizes a creative role of phenotypic plasticity in evolution. First, the effect of climate on lizard life history is largely a passive result of how thermal opportunity constrains activity patterns rather than an evolved adaptation. However, such non-adaptive plasticity apparently can be an important source of variation in selection on seasonal sex ratio adjustment and, hence, sex-determining mechanisms across species' distributions. Second, the observation that stressfully high or low temperatures have a causal effect on sex determination also in vertebrates with GSD suggests that temperature-induced developmental plasticity can simultaneously expose variation in sex determination and cause novel selection on this variation, thereby greatly facilitating evolutionary divergence in sex determination. If so, transitions in sex-determining systems may only require minor secondary modifications, loss, or gain of regulatory elements involved in gonad differentiation, suggesting substantial scope for interchangeability between genetic and environmental determinants of sex.

The evolution and suppression of male suicide under paternal genome elimination

Laura Ross & Ido Pen (in collaboration with David Shuker, University of St. Andrews, UK)

Different genetic systems can be both the cause and the consequence of genetic conflict over the transmission of genes, obscuring their evolutionary origin. For instance, with paternal genome elimination (PGE), found in some insects and mites, both sexes develop from fertilized eggs, but in males the paternally derived chromosomes are either lost (embryonic PGE) or deactivated (germ line PGE) during embryogenesis and not transmitted to the next generation. Evolution of germ line PGE requires at least two transitions: (1) elimination of the paternal genome during spermatogenesis; (2) deactivation of the paternal genome early in development. So far, hypotheses for the evolution of PGE have mainly focused on the first transition. Interestingly, it has been shown in some scale insects that the second transition is triggered by maternal genes rather than the embryo's own genes. We conjectured that this may have evolved as a response to suicidal tendencies of the embryo's paternal genes and we developed models to investigate whether this argument is sound.

How could paternal genes be selected to favour their own destruction? Two observations are crucial to see this. First, in species with PGE paternal genes of males are never transmitted to the next generation – hence they have 'nothing to lose'. Second, paternal genes in males may gain inclusive fitness if the dead bodies of males can be 'recycled' to some extent and thereby improve their sisters' survival prospects. Thus, in order for male suicide to evolve it seems necessary that there is some degree of sib competition over vital resources. Benjamin Normark noted that most taxa with PGE do indeed have strong levels of sib competition. However, he also observed that these species typically have high levels of sib mating and are therefore quite inbred. At first glance, one might expect inbreeding to counteract the spread of paternal male suicide as it can lead to increased relatedness between the maternal and paternal genome of an individual. However, inbreeding also increases relatedness between sibs, which might promote male suicide. To make matters even more complicated, a life history with inbreeding and sib competition tends to induce selection for female-biased sex ratios, thus increasing the reproductive value of individual males, which might be an additional obstacle to the evolution of male suicide. Clearly, a formal model is required to investigate the balance of these opposing effects.

In our model (Ross, Shuker & Pen, *Evolution*, 2010), we considered the fate of a partially suicidal gene that is expressed in males by the paternally inherited half of their genome. Specifically, the gene causes a probability x of suicide, and we let the value of x evolve. A parameter b was introduced as a measure of the recycling efficiency (between 0 and 1) of dead males. We allowed for some degree of inbreeding by assuming that the population is subdivided in standard-sized patches of n mated females whose offspring mate randomly on their natal patch followed by dispersal of newly mated females. These ingredients were incorporated in an analytical kin selection model to study what level of suicide, if any at all, is expected to evolve depending on recycling efficiency (as determined by b) and the level of inbreeding (as determined by n).

Some results are shown in Figure 9. Male suicide can evolve, and unless some inbreeding occurs, 100% suicide evolves and population extinction will be the result, regardless of the efficiency of recycling. If maternally-controlled sex ratios are allowed to co-evolve, male-biased primary sex ratios are possible, in contrast to standard models that predict female-biased sex ratios for populations with inbreeding. Maternal suppression of male suicide is always selected for, leading to complete suppression of male suicide, even if dead males are recycled with 100% efficiency.

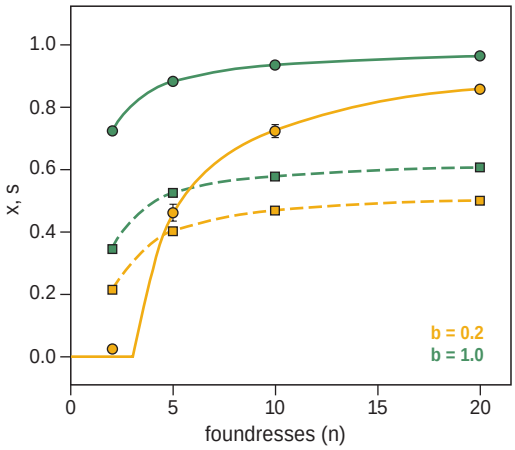

Figure 9. Male suicidal tendency (x) and sex ratios (s) predicted by the model, as a function of number of females per patch (n) and efficiency (b) of recycling dead males. Curves indicate predictions of analytical kin selection model and symbols with error bars are means ± standard deviation of 10 individual-based simulation replicates. Note that inbreeding (lower n) selects against suicide, while higher male recycling efficiency favours male suicide.

The evolution of male fertility

Leif Engqvist

Fertilization success is decisive for how many offspring a male will sire. Traits increasing the competitive ability of male sperm should be strongly favoured by selection, eliminating variation in sperm number and type. It is therefore surprising that differences in male fertility are widespread in nature, both between and within species. How can we explain this variation from an evolutionary point of view? In my recent research, I have focused on some of the open theoretical questions associated with the evolutionary processes influencing male fertility. Here I describe the results from two recent projects.

Sperm competition (*i.e.* the competition between sperm from two or more males for the fertilization of a given set of ova) has been identified as a strong selective force shaping the evolution of many male reproductive traits. In the first project, I investigated whether and how sperm competition should affect sperm viability. The longer a male's sperm can survive, the longer they can participate in the competition for egg fertilization. Following the logic that male sperm traits will be more important at high levels of sperm competition, it has repeatedly been suggested that the benefit of high sperm survival should increase with an increasing level of sperm competition. However, there is at present no theoretical analysis of this hypothesis. I therefore used an evolutionary game-theory approach to find the evolutionarily stable strategy (ESS) with respect to male investment in sperm viability.

The most important result of this analysis was that the ESS sperm mortality rate will increase with an increase in female mating rate, thus leading to less viable sperm (Figure 10). Hence, in matings systems, where females mate with many males, sexual selection is predicted to drive sperm viability to lower levels compared to mating systems where females mate less frequently. This

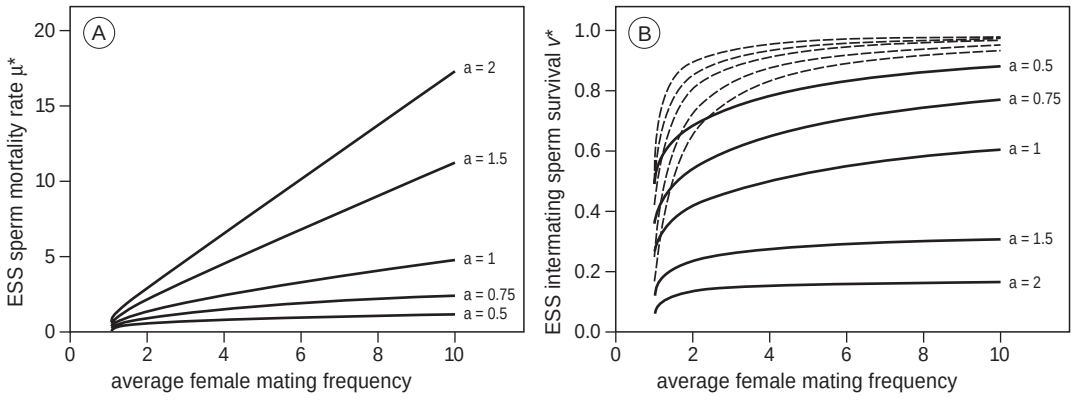

Figure 10. Effect of sperm competition on the evolutionarily stable level of sperm viability. Results showing A) the ESS sperm mortality rate μ^* in relation to average female mating frequency for different values of the parameter *a*, which defines the cost of producing long-lived sperm. In B) μ^* is expressed as the proportion of sperm surviving between female matings. The dashed lines depict expected values if sperm mortality would remain at values predicted for minimal levels of sperm competition.

might at first glance seem as a highly unreasonable result. How can we make more sense out of this prediction? If females mate frequently, males are very likely to face sperm competition. Investing in highly viable sperm is analogous to investing in future fertilization opportunities. However, when females have a high remating rate, these future fertilization chances will be decreasingly small because a male's sperm will compete against an increasing number of rival sperm. Hence, the more males a female will mate with in the future, the less beneficial it will be to invest in viable sperm that can fertilize eggs in the future. On the other hand, if females mate at a very low frequency, most male matings will be with virgin females. Sperm will thus be less important for male reproductive success. Here the only reason to invest any excessive energy in sperm is for them to survive long enough until the female remates and sperm competition does occur. These novel results should have strong impact on future sperm competition studies and will also have strong implications for our understanding of the evolution of female mating rate.

In the second project, I investigated how intrinsic differences in male fertility should affect a male's investment into any given ejaculate. Variation in male fertility is common and, for example, caused by selfish genetic elements or the accumulation of deleterious mutations with male-specific effects in mitochondrial DNA ('mother's curse' mutations). Yet, most theoretical analyses of sperm competition assume that all males within a population have the same fertility. I dropped this assumption, in order to answer the question: should males that are intrinsically less fertile compensate and invest more in sperm traits, or instead concentrate investment on traits affecting mating success?

My theoretical analyses have revealed that differences in male fertility can have large effects on overall male sperm investment. In a first model, I assumed that males do not know their intrinsic fertility and, hence, cannot make their ejaculate investment dependent on their own fertility. For this scenario, I could show quite generally that in populations with larger variation in male fertility, fewer resources should be spent on ejaculates. The reason for this is that as inter-male fertility differences become larger, fertilization success will be less influenced by ejaculate investment; instead intrinsic male fertility will dictate sperm competition success. In contrast, when all males are equal, ejaculate investment is the only influencing factor, and males are expected to invest more. In a second model, males were able to allocate resources on sperm investment dependent on their own fertility. In this scenario, the model predicts that less fertile males should compensate their disadvantage by investing more in sperm competition, unless differences between males are very large.

The evolutionary ecology of a mutualism

Aniek Ivens, Ido Pen & Franjo Weissing (in collaboration with Koos Boomsma, University of Copenhagen, Denmark; Daniel Kronauer, Harvard University, USA)

Mutualisms, interactions in which individuals of different species cooperate, are widespread throughout nature. Examples include plants and their pollinators, cleaner fish and their clients and light-producing bacteria that inhabit bobtail squids. In fact, in the form of mitochondria, mutualism stood at the basis of life as we now it. Recently, mutualisms also are increasingly recognized for their importance to ecosystem diversity and stability. Consequently, an increasing number of studies aims to unravel details of specific interactions such as the natural history of the mutualisms, costs and benefits for the partner species involved and the effects of these mutualisms on their ecosystem.

Despite this increasing focus on mutualisms, their evolution and evolutionary stability are still poorly understood. How does cooperation between different species come off the ground? And once a mutualistic interaction has been established, how does it stay stable over evolutionary time? Where two species live this closely together, the interests of the partner species are expected to never be fully aligned, potentially leading to conflicts disrupting the interaction. The many stable mutualisms we see in nature are therefore possibly results of ongoing mutual exploitation by the partners with net benefits for both, rather than harmonious cooperation. In our project, we aim to shed light on these questions using a combined theoretical and empirical approach. Here we will focus on the empirical part of the project.

Cultivation mutualisms are mutualisms in which a host species promotes the growth of the symbiont species which it consumes or on which it relies for food. Classical examples of cultivation mutualisms are algae-growing damselfish and fungus-growing ants. Both ants and damselfish actively grow their symbiont species as a crop for food in especially constructed gardens. Similarly, root-aphid tending ants keep aphids as cattle in aphid-chambers in their nest. The ants rely for their sugar consumption on the honeydew the aphids produce and in return the ants provide the aphids with housing and active protection against predators. It is the latter system of aphid tending ants that is the focal system of our research. Like other mutualisms, also cultivation mutualism can be prone to destabilizing conflicts of interests between the partner species. Three major potential sources of conflict are energy allocation of the symbiont towards sexual reproduction, competition with other symbionts (due to high within-host symbiont diversity) and dispersal. This energy allocation may compromise symbiont productivity and it would therefore be in the host's interest if the symbiont invested all energy in productivity. However, the symbiont might benefit from sexually reproducing, outcompeting other symbionts or dispersing and might prefer to allocate at least some energy to these. How are these conflicts resolved in an aphid-ant mutualism? What mode of reproduction do the symbiont aphids have? What is their degree of diversity (and thus competition) and to what extent do they disperse independently of the ants?

We investigated these questions in a particularly interesting cultivation mutualism: the interaction of the yellow meadow ant *Lasius flavus* and the root-aphids it tends in its nest. More than ten different species of root-aphids have been found in *L. flavus*

nests, where they feed on the roots of various grasses. The ants are said to be dependent on the honeydew produced by these aphids as their sole sugar source and are also known to occasionally prey on the aphids. We conducted a population genetic study on these aphids, focusing on the four most common and abundant species: *Geoica utricularia, Tetraneura ulmi, Forda marginata* and its sibling species *Forda formicaria*. To this end, we sampled aphids from ant mounds along a transect on the island of Schiermonnikoog. About 500 aphids were collected and genotyped with species-specific sets of microsatellite markers, developed by us prior to this study.

The results show that all four focal aphid species reproduce predominantly clonally in the sampled population. Year-round clonal reproduction had been described before for *G. utricularia* and both *Forda* species, but it was surprising that also *T. ulmi* had apparently lost sexual reproduction. In order to gain insight in aphid diversity and dispersal levels, we mapped the distribution of clonal lineages

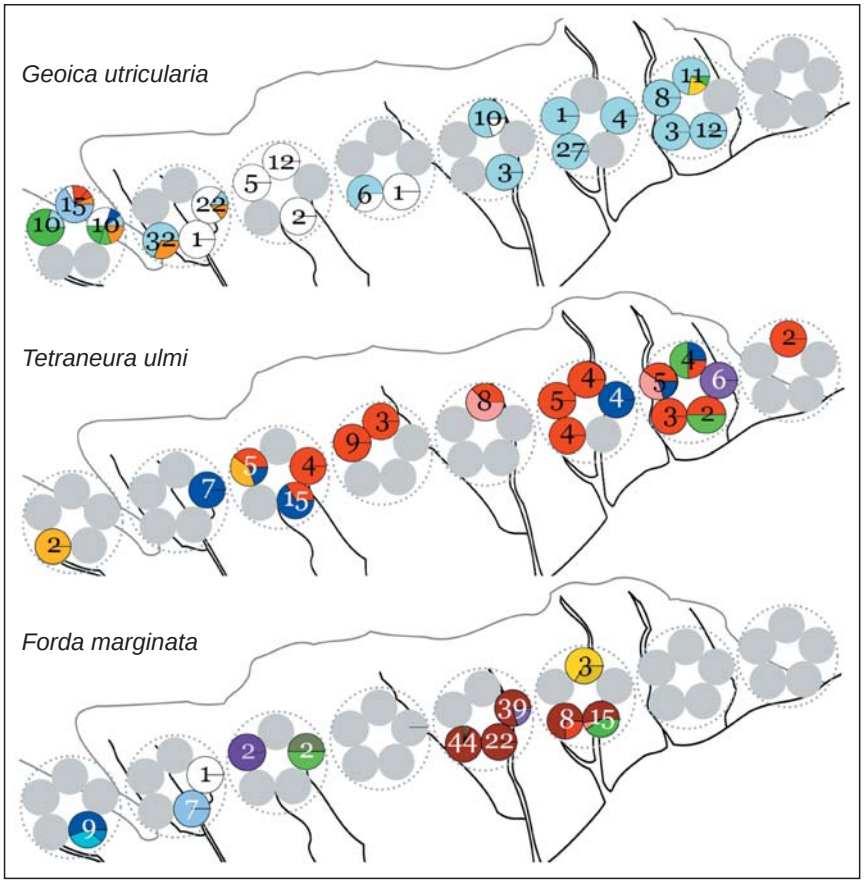

Figure 11. Distribution of clonal lineages of three species of root-aphids showing a mutualistic relationship with yellow meadow ants: *Geoica utricularia* (top), *Tetraneura ulmi* (middle) and *Forda marginata* (bottom). Dashed circles refer to transect locations, filled circles refer to sampled ant mounds. Colours show the proportion of the total sample (numbers given) for that species in an ant mound belonging a particular genotype, colours belonging to the same colportage belong to the same clonal lineage. Grey circles refer to ant mounds where the focal species was absent.

over the island and over and within ant mounds (Figure 11). All four species have a single predominant clone to which the majority of the collected aphids belongs. A couple of clones occur at several transect locations, but only one clonal lineage occurs along the whole transect. This indicates that aphids do occasionally disperse, but that this dispersal is very limited. Aphids did often occur in monocultures (in terms of species as well as clonal lineages) but not exclusively. The scope for between-aphid competition is further reduced by the aphid species and clonal lineages occurring spatially segregated in the ant mounds. Lastly, the same aphid clonal lineages seem to stick with the same ant mounds over time as no major turn over in aphid composition was observed over years.

To conclude, in this system the aphid symbionts do not reproduce sexually, do occur at low degrees of (genetic) diversity and show low levels of dispersal. These symbiont characteristics show remarkable similarity to what is observed in fungus-growing ants. Also their symbiont fungus reproduces clonally, disperses independently to a limited extent and occurs as a monoculture in the garden. Whether we are dealing here with conflict resolutions universal for cultivation mutualisms will be further investigated in the theoretical part of this project.

Modelling the hydrodynamics of swimming by fish

Daan Reid, Hanno Hildenbrandt & Charlotte Hemelrijk (in collaboration with Johan Padding, Catholic University of Louvain, Belgium)

The swimming of fish has fascinated scientists for centuries. Only recently has it become possible to study the hydrodynamics of swimming experimentally. These empirical measurements of hydrodynamics are difficult and time-consuming, and are inaccurate as regards the measurement of the forces of swimming, *i.e.*, thrust and drag. As a consequence of this a number of assumptions and theoretical predictions have not yet been tested. We used a computer model to do so. We chose the so-called 'Multiparticle Collision Dynamics' method, in which hydrodynamic features emerge from the movement and collision of millions of particles. In view of its flexibility (e.g., its independence of a restrictive spatial grid) and its computational efficiency, this method is particularly suited to model the hydrodynamics of moving organisms. We investigated three common assumptions, namely that 1) only the kinematics of the tail tip causes thrust during undulation; that 2) the efficiency of swimming may be estimated by the slip ratio U/V between the forwards swimming speed U and the rearwards speed of the body wave V; and that 3) preventing individuals from accelerating (forwards or sideways) does not affect their swimming.

Before applying the model to swimming fish, we validated it for the case of a flapping cross-section of an insect wing, because detailed empirical data of drag and lift are available in this case. We measured the resisting forces in the flapping direction (drag) and perpendicular to it (thrust) and found good agreement with theoretical and experimental results. Especially of note is the fact that the model could reproduce the peaks in the forces as the wing reverses direction and encounters its own wake, and as the wing rotates strongly.

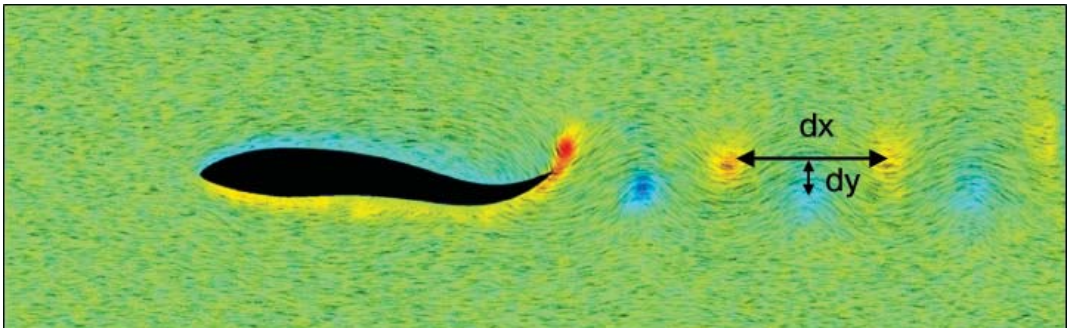

Figure 12. Wake structure of a swimming fish. *dx* and *dy* are distances between vortices in the wake. Colour indicates direction of vorticity, with blue indicating clockwise and red counter-clockwise rotation.

When applied to undulating fish, our model produces, like in real fish, a wake consisting of counter-rotating vortices with a jet that zigzags between them, a so-called reverse von Kármán wake (Figure 12). Regarding the area of the body that generates thrust during undulation, our model shows that – contrary to previous theoretical predictions – not only the tail tip, but the entire rear half of the body produces thrust (Figure 13). Similarly to real fish, our modelled fish move faster as their tail-beat frequency increases (Figure 14A). A fish with faster tail-beat produces both more (wasted) power sideways and a stronger (useful) force forwards (thrust, Figure 14B,C). Further, at faster tail-beat the slip ratio U/V increases (Figure 14D). Slip ratio is generally supposed to reflect efficiency; accordingly efficiency should increase with the tail-beat frequency too. However, the opposite is found in our model: the 'Froude efficiency' $\eta = TU / (TU + P_s)$ (where T is thrust, and P_s is the power exerted sideways) decreases as the fish beat their tail faster (Figure 14E).

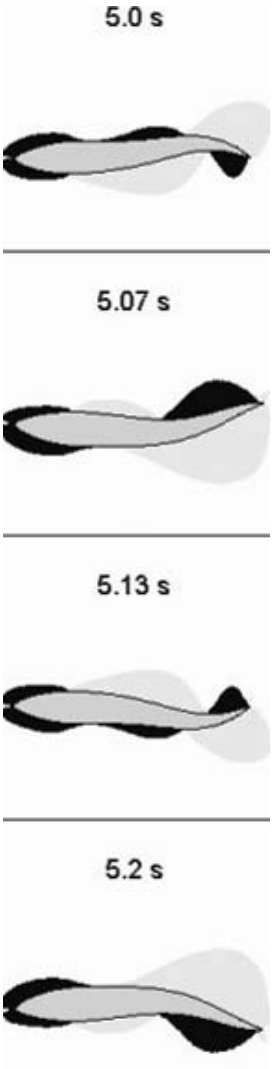

Figure 13. Drag and thrust on the skin of the fish over time, for tail-beat frequency of 3.8 Hz, for fish that are free in to accelerate. Black indicates drag, gray thrust. Force areas are composed of lines perpendicular to the skin, with the length of the line indicating the relative size of the force on that segment of the skin.

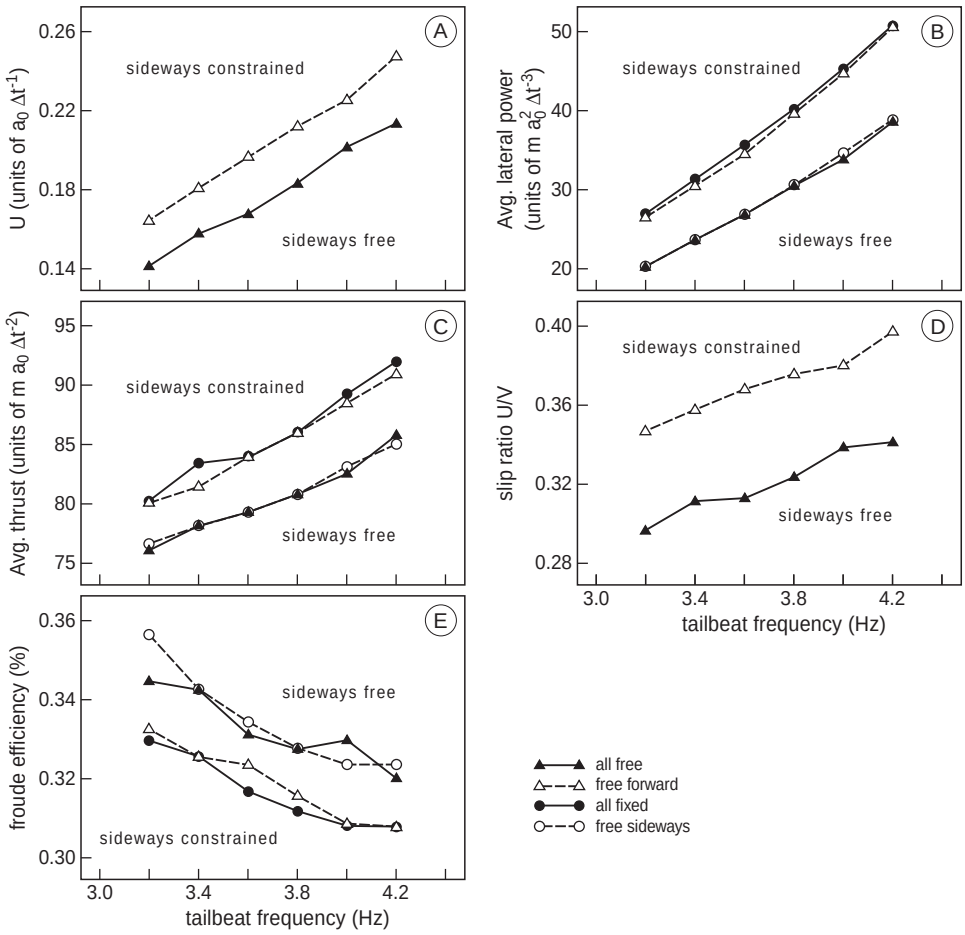

Figure 14. Effect of tail-beat frequency on different traits. A) Equilibrium speed forwards. B) Average lateral power (in simulation units) exerted by the fish. C) component of average forwards thrust (in simulation units). D) Slip ratio *U/V*. E) Froude efficiency. Fish are either 'constrained' or 'free' in their acceleration forwards and sideways.

In sum, our results demonstrate the suitability of the Multiparticle Collision Dynamics model for the modelling of hydrodynamics of swimming fish at moderate to high Reynolds numbers. Further, we conclude that fish produce thrust through a larger area of their body than commonly assumed, that the slip ratio *U/V* does not correctly estimate the efficiency of swimming, and that sideways constraint of the acceleration of modelled fish will result in speed and efficiency of swimming that correspond to those found in freely swimming fish with a higher tail-beat frequency.

Swarming behaviour through self-organisation

Charlotte Hemelrijk, Hanno Hildenbrandt (in cooperation with Eize Stamhuis and José Reinders, Ocean Ecosystems; Claudio Carere, Instituto Superioire di Sanità, Rome)

Models of self-organisation have shown that coordination among moving individuals leads to stable swarming behaviour. Although recently such models have been studied extensively, even in physics, the extent to which their patterns of swarming really resemble empirical data is seldom verified. This is in part a consequence of the difficulty to measure the three-dimensional positions of individuals in a swarm. Here we show for two cases where these difficulties were overcome how comparison of model and empirical results may increase our understanding of patterns of swarming by animals.

First, fish schools are known to be oblong in shape. Our previous models (Hemelrijk & Hildenbrandt, *Ethology*, 2008) show that such a shape develops automatically in a model in which individuals move and have three tendencies, 1) to avoid colliding with others if they are close, 2) to align with others at intermediate distances and 3) to approach others further away (Figure 15A). These models also showed that if schools are larger, individuals

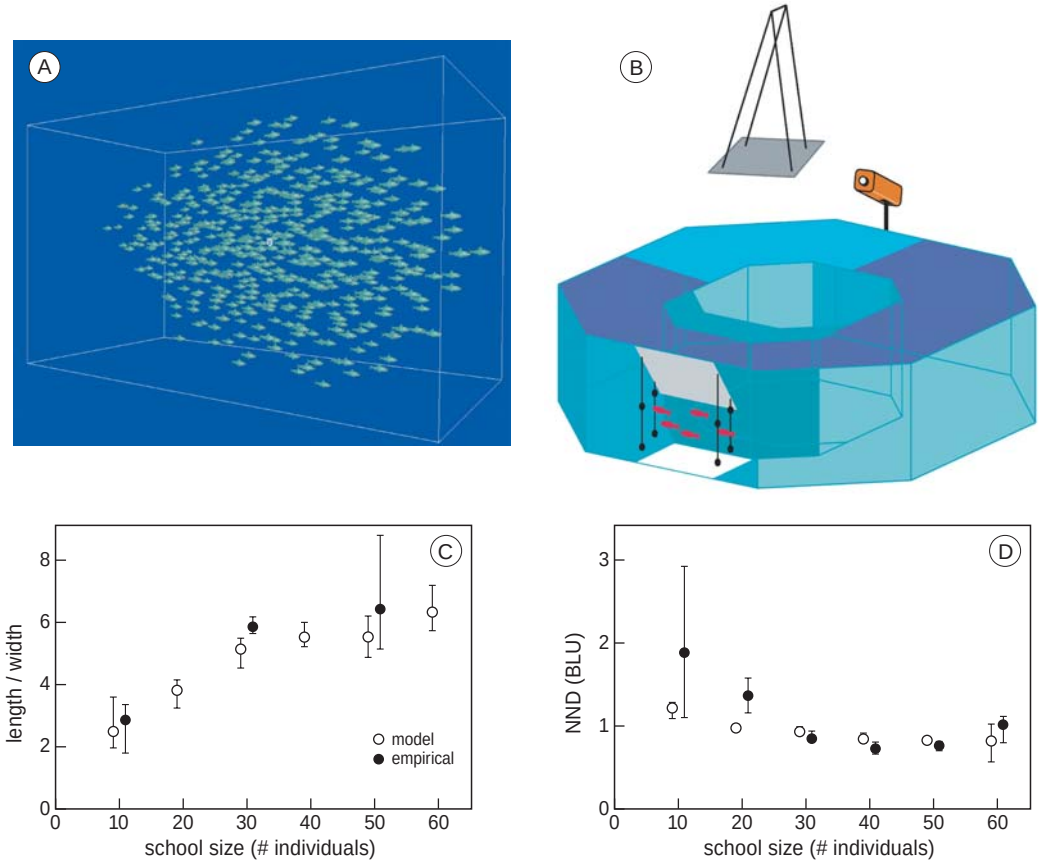

Figure 15. Top: A) Model of a fish school, B) Octagonal tank. Bottom: The effect of C) oblong shape (length/width) and D) distance to the nearest neighbour on group size. Empirical data are in black, modelling data are open.

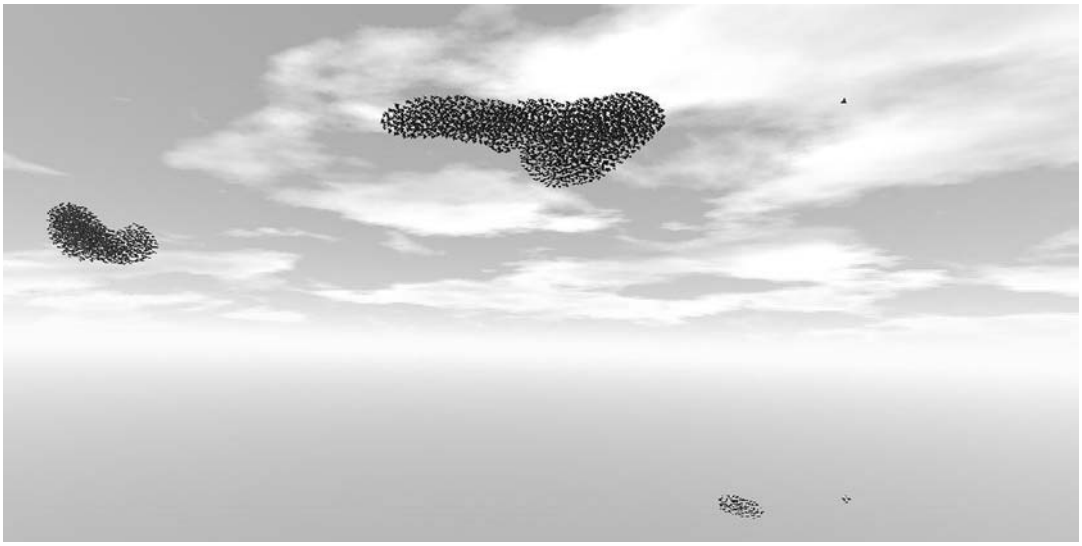

Figure 16. Flocks of starling in StarDisplay (10.000 individuals)

are closer together and schools are even more oblong. The oblong shape emerges in these models, because individuals slow down to avoid colliding with a neighbour to the front and this leaves a gap between the former neighbours. Consequently, the former neighbours move inwards. Thus, the school has become oblong. Larger schools are more oblong, because individuals need to avoid others more frequently due to the closer proximity to others in larger schools. We have tested empirically in an octagonal tank whether larger schools of real fish are also denser and more oblong. For this we measured the three dimensional locations of individual mullets in schools of 10 to 6o individuals (Figure 15B). We confirmed our prediction (Figure 15C,D). Thus, we have increased our understanding of school shape in real fish through our model-based theory (Hemelrijk *et al., Ethology*, 2010).

Second, flocks of birds are one of the most complex swarming structures known. For instance, the flocking manoeuvres of huge flocks of thousands of starlings at dawn above their sleeping site (so-called roost) are amazing in their variability in shape and density. So impressive are these flocks and the speed with which they change their shape and density, that in the 1930s flocking manoeuvres were explained as a case of 'telepathy' or 'thought transference'. Recently, the three dimensional positions of individual birds in large flocks of starlings have been quantified in flocks above Rome and we have used these data to investigate whether their flock shapes may emerge by self-organization instead.

We have shown that comparable patterns of flocking can indeed be generated in a model, called StarDisplay (Figure 16; Hildenbrandt *et al., Behavioural Ecology*, 2010). In this model individuals are steered by simple behavioural rules. They coordinate with others like in the fish model, but in contrast to our fish model, like in real birds they only mind their seven closest neighbours, they fly according to simplified aerodynamics, they roll into a turn, once outside the roost they turn around to return to it and they hardly adjust their speed. The shapes of these artificial flocks remarkably resemble those of real flocks. The aspects of the bird model that are most responsible for this resemblance appear

to be the rolling during turning, aerodynamics and the large size of the flock. When individuals roll into the turn and they turn sharply, they also move downwards, because during the turning lift has been exchanged for centripetal force. This downwards movement causes flocking shape to be highly variable. When we omit the rolling when turning, the shape of the flocks becomes extremely oblong, and the flock circles statically around the border of the sleeping site. This does not resemble real starling flocks at all.

When flocks are large, the shape is variable, because once a few individuals have crossed the border of the sleeping site, these frontal individuals may already start to return to the roost while others (at the back of the flock) are still moving outwards. Consequently, the flock compresses. When later on all individuals move towards the roost again, the flock dilates. This means that the flock is variable in its shape. StarDisplay is useful for generating new hypotheses about the causation of other traits of starling flocks too. If empirical data will confirm these hypotheses, like in the case of our fish school, we will gain 'understanding by building'.

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